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From the Department of Diagnostic Radiology (Head Aa Wagner, M D) Aarhus Amtssygehus Aarhus, Denmark

The Normal Gastric and Duodenal Rugae

Radiographic findings in non dyspeptic young persons

By

EINAR KRAG[1]

The normal radiographic appearance of the gastric and duodenal mucosae was described in 1913 and 1923, respectively, by Forssell (3, 4), who especially emphasised the autoplastic properties of the mucosal relief, which are manifested by incessant changes in the appearance and course of the folds

Rendich (7) also called attention to the variable radiographic appearance of the normal gastric mucosa However he concluded that the folds usually run roughly parallel in the direction from the cardia to the pylorus, except along the greater curvature, where they are often divided up into small folds with a more or less tortuous course The width of the folds was reported to range from 2 to 4 mm (supine film, target film distance, 22 in)

Berg (1) likewise stressed the auto plastic properties of the gastric and duodenal mucosae He stated that the width of the folds was as for a straw, or about 5 mm

It is very difficult to draw a clear line of distinction between the normal and a pathological mucosal relief because no reports on radiographic studies of the gastric and duodenal mucosae performed in a large number of healthy, non-dys peptic individuals are available in the literature (2) Such studies will, how ever be required in order to attain a more reliable evaluation of the clinical importance of the radiological concepts of gastritis and duodenitis as pre viously discussed in connection with a study of the prognostic significance of coarse irregular mucosal folds in pa tients with the ulcer disease (6)

Material and methods

The series of normal subjects studied consisted of 94 medical students (79 men and 15 women) who were all in good health without any signs of dyspepsia None of the subjects had previously suffered from gastro-

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Fig 1 Erect position film Slightly irregular mucosal folds in the fundus



Fig 2 Supine film Regular and parallel folds in the body and prepyloric region

intestinal disorders Their ages ranged from 20 to 30 years

Before the radiographic examination of the gastric mucosa each subject had swallowed 40 ml of a barium sulphate solution (Micropaque® 100% w/v) in front of the fluorescent screen Immediately after this, one exposure of the mucosal pattern in the prepyloric region and the body of the stomach was made with the subject in the dorsal position, and another in the erect position in order to visualise the relief of the fundus

The duodenal mucosa was studied radiographically in only 59 of the 94 students Each subject was given 150 ml of the contrast medium just mentioned 5 min before the examination Under fluoroscopic control, one exposure of the duodenum including the bulb was made in the erect position and one in the dorsal position

The filter film distance was about 70 cm = 27 5 in

Results

Gastric mucosa

In all the subjects studied the fundus revealed slightly irregular mucosal folds (fig 1)

In the body and prepyloric region the course of the folds was largely regular and parallel to the curvatures (fig 2), with the exception of 6 cases in which a slightly irregular course was observed in the films taken in the dorsal position (fig 3)

In four subjects, the mucosal pattern could not be assessed because of ingested food

The width of the mucosal folds was less than 5 mm in all subjects but one, in whom the films taken in the dorsal



Fig 3 Supine film Slightly irregular folds in the body of the stomach



Fig 4 Supine film Regular folds of the bulb and feather boa pattern in the remaining part of the duodenum

position showed folds of a width of up to 7 mm but the pattern was also regular in this case

None of the individuals studied showed concurrent existence of coarse irregular mucosal folds

Palpation with a glove under fluoroscopic guidance did not reveal any rigidity of the folds

The peristaltic activity was not increased

Duodenal mucosa

All the subjects studied showed a normal duodenal bulb without signs of deformity or irritability

The folds of the bulb were regular and the remaining part of the duodenum revealed the characteristic feather boa pattern (fig 4) except in two cases in



Fig 5 Supine film Hypotonic duodenum with regular folds but no feather boa pattern

which the duodenum was rather hypotonic but with a regular mucosal pattern (fig 5) All folds observed were less than 5 mm in width

The study failed in three subjects

The peristaltic activity was not increased, and palpation under fluoroscopic guidance did not show any signs of rigidity of the folds

Discussion and conclusions

The series of normal subjects considered here does not quite fulfil the statistical *requirements made on a random sample* because they were selected on the basis of four criteria a) none of the subjects suffered from dyspepsia, b) all were medical students, c) the study was not accomplished in all cases, and d) all belonged to the age group 20—30 years The reason for the selection of subjects in the third decade of life was twofold Firstly, it is difficult to obtain a representative section of normal subjects of all age groups, secondly, it is of particular interest to study individuals in the third decade of life because previous studies (5) have shown that this age group contains a large number of patients with the pseudo-ulcer syndrome, i e symptoms of the ulcer disease but no demonstrable ulcer Many of these patients present coarse, irregular folds

From the investigation reported above it must therefore be concluded that in non-dyspeptic individuals aged from 20 to 30 years the gastric and duodenal mucosae show a regular mucosal pattern with non rigid folds which are less than 5 mm in width

Summary

The morphology of the gastric mucosa was studied radiographically in 94 healthy, non dyspeptic medical students aged from 20 to 30 years The morphology of the duodenal mucosa was also studied in 59 of these subjects

Both the stomach and the duodenum revealed a regular mucosal pattern with non rigid folds which were less than 5 mm in width

Addendum

The student who showed folds of a width of up to 7 mm has now 5 months later developed typical ulcer-dyspepsia

Acknowledgement

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From the Medical Clinic B (Renal Clinic) and the Department of Surgery University Hospital Lund Sweden

Hypersecreting Villous Rectal Papilloma Leading to Excessive Electrolyte-fluid Losses and Acute Renal Failure

By

PER ERLANSON, BENGT LINDQVIST and GORAN LUNDH

In cases of villous papilloma in the colon and rectum discharge of blood and mucus in the stools is often noted A less well-known fact is that the increased secretion of mucus from these tumours can sometimes give rise to diarrhoeal disorders which may persist for many years and, in turn lead to electrolyte fluid depletion Some 30 such cases of hyper secreting villous papilloma have been described in the literature the first one by McKittrick and Wheelock in 1954 (6) Diagnostic and therapeutic problems associated with this form of tumour have been discussed by, for instance, Shamblin et al (8) and Davis et al (1)

Prerenal uraemia always occurs in these cases, but acute renal failure resulting from the electrolyte disturbances does not seem to have been reported earlier The diagnostic and therapeutic problems of hypersecreting villous papilloma in the rectum are illustrated by the case presented here, in which the condition was complicated by acute renal failure, among other disorders

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Case report

(Fig 1 shows the clinical course)
A 55 year old furniture dealer had for many years had diarrhoea intermittently mostly in connection with anxiety and stress On July 19 1963 (day 1) he became ill with diarrhoea He passed five or six loose stools daily for 2—3 days and thereafter they became watery and also occurred at night during sleep His abdomen became moderately distended but he had no pain His appetite was poor and he took only water and tea He was admitted to a hospital for infectious diseases on day 8 His general condition was then moderately poor and he showed signs of dehydration His heart rate was 120 beats/min and B P 110/70 mm Hg Hb was 118 % white cell count 17 400/mm³ serum albumin 8 9 % standard bicarbonate 24 mEq/l serum sodium 116 mEq and serum potassium 2 3 mEq/l chlorides 55 mEq/l phosphate 15 9 mg/100 ml Non

protein nitrogen (NPN) measured 4 days later was 190 mg/100 ml ESR was 6 mm/hr, urinary output 500—1,000 ml/24 hours During the first few days he was given 3,000—5 000 ml of fluid, partly liquids by mouth, partly carbohydrate solutions parenterally On days 11 and 12, he also received a total of about 500 mEq of sodium chloride and 150 mEq of potassium chloride

Because of increasing uraemia he was transferred to this clinic on day 12 On admission he was drowsy but oriented as to time and space The subcutaneous tissues were slightly reduced but had normal tone There were no cerebral or other signs of fluid retention with intracellular oedema His abdomen was highly meteoric Serum sodium was 107 mEq potassium 2 4 mEq and chlorides 76 mEq/l Standard bicarbonate was 11 8 mEq/l NPN 200 mg/100 ml Hb 14 7 g/100 ml, white cell count 17 900 and thrombocyte count 240 000/mm^3 He had a slight bilateral pleural effusion Urinary output was about 700 ml on the day of admission The hypoelectrolytaemia and uraemia necessitated administration of increased amounts of electrolytes — 70 mEq of potassium and 80 mEq of sodium were added on the day of admission — and dialysis treatment on the next day with potassium rich dialysis fluid in the machine But his electrolyte values did not rise and his condition deteriorated further with increasing meteorism and falling blood pressure

Pneumonia and respiratory insufficiency due to the severe meteorism necessitated tracheotomy and respirator treatment Since paralytic ileus could not be excluded the colon was examined by X ray, despite the patient s poor general condition The examination which could not be completed because of his poor state showed no evidence of mechanical obstruction but at the transition between the sigmoid flexure and the rectum the intestinal wall seemed to be pathologically changed

During daily administration of hypertonic solutions with very large doses of potassium (maximum 400 mEq 24 hours) and sodium (maximum 1,100 mEq 24 hours), the serum electrolyte level became normal and the intestinal atony decreased gradually Despite this correction urinary output fell to 100—300 ml /day, urinary NPN was low (less than 500 mg/100 ml), and blood NPN rose rapidly, indicating acute renal failure He was therefore dialysed eight times up to day 30

He usually passed 2 or 3 l of watery stools per day and between 1/2 and 1 l of fluid was discharged daily through a gastric tube Besides electrolytes, albumin was lost in great amounts, which were replaced by parenteral albumin The parenteral administration of hypertonic saline solutions gave rise to multiple thrombophlebitis with skin necrosis, and these lesions became infected

The patient s general condition gradually improved, daily urinary output increased from about 100 ml to about 250 ml, while the NPN level in the blood rose more slowly Further investigation of his intestine, especially with a view to the suspected change in the colon, had been planned but was postponed, because at the 7th dialysis on day 26 he had profuse gastrointestinal haemorrhage accompanied by severe shock with deep coma unmeasurable blood pressure, unrecordable pulse, inaudible heart beats and only a few short respirations per minute In response to external heart massage respirator treatment, prompt administration of sodium bicarbonate Rheomacrodex®, blood and noradrenaline his blood pressure became measurable and within about 10 min spontaneous movements returned Further administration of about 2 l of blood and Macrodex® led to improvement and on the next day he was in good general condition and had a normal blood pressure The improvement continued up to day 30 and daily urinary output rose to between 1 and 2 l

As from day 31, he had temperature rises up to 39° C urinary output decreased to 400—500 ml and NPN rose to a maximum of 138 mg/100 ml Blood culture on day 29 had yielded sparse growth of Ps pyocyanea which also grew in wound secretion and tracheal secretion During treatment with

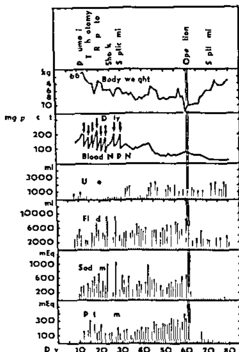


Fig 1 Schematic representation of the case history



Fig 2 Villous rectal papilloma measuring 5 × 8 × 15 cm resected from upper part of rectum and lower part of sigmoid

colimycin and streptomycin his temperature fell and daily urinary output increased to between 1 500 and 2,500 ml NPN fell spontaneously to about 50 mg/100 ml

The great losses of fluid from the intestine and through the gastric tube showed no tendency to decrease As will be seen from fig 1 he was given 1,400—9 250 (mean 5 200) ml of fluid 150—1 100 (mean 460) mEq of sodium and 70—400 (mean 200) mEq of potassium per day to maintain normal electrolyte values

As the patient s general condition improved sigmoidoscopic examination was performed on day 54 It revealed a large papilloma situated about 10 cm from the anus On day 61 resection was performed of the upper part of the rectum and the lower part of the sigmoid which contained a villous rectal papilloma measuring 5 × 8 × 15 cm (fig 2) In view of the patient s poor condition a primary anastomosis was not established but the rectum was invaginated and brought in under the peritoneum and a sigmoidostomy was set up *Histological diagnosis*, a villous papilloma with in part marked cell atypia but without conclusive evidence of malignant change

The patient tolerated the operation surprisingly well Immediately after it the discharge from the intestine and through the gastric tube diminished and the stools became normal During the next few days his temperature rose to 39 C and he became moderately stuporous Blood cultures on days 73 and 75 yielded growth of a Candida species which also grew in cultures from faeces and urine During treatment with Amphotericin B® the blood cultures became negative Thereafter the patient s general condition improved gradually On day 90 he was able to be ambulant and after another week he could walk a good distance in the hospital corridor On day 113 he was sent back to the general hospital for further care

Fig 3 shows the levels of NPN potassium sodium and chlorides in serum urine diarrhoeal fluid and gastric fluid The NPN level was highest in the urine distinctly lower in the gastric fluid and serum, and lowest in the diarrhoeal fluid The level of potassium in the diarrhoeal fluid was very high and of he same order of magnitude as in the urine much lower in the gastric fluid and low in

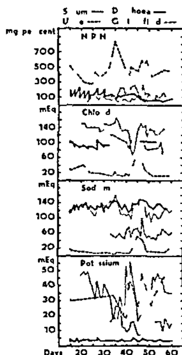


Fig 3 Concentrations of NPN potassium, sodium and chlorides in serum urine diarrhoeal fluid and gastric fluid

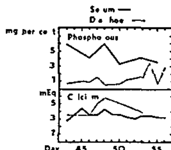


Fig 4 Concentration of calcium and phosphorus in serum and diarrhoeal fluid

serum The sodium level was approximately equally high in serum and diarrhoeal fluid *much lower in the gastric fluid and lowest* in the urine As regards the chlorides the levels were higher in the diarrhoeal fluid *than in serum and gastric fluid and low in* the urine

Fig 4 shows that the concentration of calcium in the diarrhoeal fluid was slightly higher than in serum, whereas that of phosphorus was lower

The electrolyte values are summarized in table I The diarrhoeal fluid, in comparison with serum, contained on an average about ten times more potassium, 1 1/2 times more chlorides and calcium an equal concentration of sodium, 1/3 that of NPN and 2/3 of phosphate

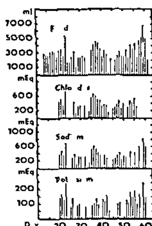
Fig 5 shows the estimated intestinal losses of fluid potassium sodium and chlorides The amounts of the discharges that could be measured are also set out The intestinal losses of fluid as well as of electrolytes varied greatly in amount from day to day

Comments

Patients with hypersecreting villous rectal papillomas tend to have diarrhoea of obscure nature, lasting for as long as 10—15 years, in Shn tka et al s series

TABLE I Levels of sodium potassium chlorides calcium phosphates and NPN in serum and excretions (mEq/l)

	Serum	Urine	Gastric fluid	Diarrhoeal fluid
Sodium	106—147	5—61	41—100	97—160
Potassium	2—4 9	4 5—53	6—19	16—56
Chlorides	55—107	10—75	75—120	114—165
Calcium	2 5—4 1			3 5—5 8
Phosphates (mg %)	3 3—5 9			0 6—3 5
NPN (mg %)	49—232	312—822	72—249	32—165



F g 5 Estimated losses of fluid potass um sod um and chlorides

(9) for an average of 5 4 years, before the electrolyte depletion ets in In our patient, the slight diarrhoea over the past years could have been a manifestation of increased secretion from his rectal papilloma The sudden and heavy electrolyte depletion may have been due mainly to greatly increased losses from the intestine Reduced fluid intake owing to nausea by Mayfield and Milner (5) believed to have been present in their case may have played a part In cases described by others the excessive losses were ascribed to purgation (4) or profuse sweating during heat waves (2)

The cause of the excessive secretion in our case can have been a mild gastro-enteritis which increased the peristaltic movements of the intestine and also stimulated the secretion of mucus from the tumour epithelium The disturbances of the electrolyte fluid balance were aggravated as for the first few days the losses were replaced by only fluid and carbohydrate solutions parenterally

The electrolyte levels in serum, urine gastric fluid and diarrhoeal fluid in our case were followed for an exceptionally long period in the diarrhoeal fluid for about 40 days and in the gastric fluid for about 30 days The electrolyte values found for the diarrhoeal fluid agree well with those reported by Shnitka et al (9) The potassium concentration 16—56 mEq/l, was higher than that found by Randall (7) in small intestinal secretion collected with a Miller Abbot tube and in ileostomy and caecostomy material The same holds for the chloride values whereas the sodium concentration was of the same order of magnitude as in Randall s cases

The levels of electrolytes, especially potassium in the gastric fluid and the diarrhoeal fluid as well as in the urine varied markedly In Randall s cases there were also great variations in the electrolyte levels of digestive tract fluid The changes in the concentrations of sodium and potassium in the gastric and diarrhoeal fluids can, to some extent, mirror a varying effect of corticosteroids which according to Goulston et al (3), and others affect the sodium/potassium ratio in secretions from the small bowel Fig 4 suggests some parallel between the urinary and diarrhoeal potassium and an inverse relationship between sodium and potassium in intestinal secretion which would fit well with an effect of corticosteroids

The kidney disorder of acute renal failure type in our case may have been due to the electrolyte fluid disturbances together with a toxic component It made repeated dialyses necessary in this case

The diagnosis of hypersecreting villous papilloma is often missed, even in cases where rectal palpation has been done The reason for this is that these papillomas are so soft that they cannot be felt with the finger In our case the papilloma could not be felt with certainty until its presence had been established by sigmoidoscopic examination Unfortunately, in many cases the patient s general condition is so poor that more thorough exploration of the rectum is not carried out Sigmoidoscopy with the patient lying in the left lateral position is possible, however, even in patients whose general condition is very bad If this examination had been made at an early stage in our case, the operation would probably have been performed sooner But the patient for the first 40 days was in such poor general condition that an operation would have involved great risks

Summary

Villous papillomas in the rectum and colon usually discharge mucus In rare cases this secretion may be so profuse that it results in electrolyte fluid depletion In our case the electrolyte fluid losses became excessive and led to renal failure, which has not been described earlier Because of the stormy course, the patient was haemodialysed eight times and enormous amounts of electrolytes and fluid were given before the correct diagnosis was established and the tumour removed The profuse secretion from the tumour during two months permitted extensive studies of the electro lyte content and comparisons with other body fluids

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From Department of Clinical Physiology and Department of Medicine II University of Goteborg, Goteborg Department of Medical Microbiology and Clinical Physiology University of Lund Lund Sweden

Studies on the Histamine Metabolism and the Complement System in Hereditary Angioneurotic Edema

By

G Granerus, L Hallberg, A B Laurell and H Wetterqvist

The interest for the pathogenesis of this often dramatic and peculiar disease has been greatly stimulated by recent observations of plasma protein abnormalities in patients with the disease It was firstly shown by Landerman et al in 1962 (10) that patients with hereditary periodic angioneurotic edema (HANE) have a decreased amount of an inhibitor normally present in plasma, of a globulin permeability factor (kallikrein) In 1963 it was then shown by Donaldson and Evans (4) that patients with this disease lack the normal plasma inhibitor of C 1 esterase Recently it was reported that in two patients with hereditary periodic edema there was a marked increase of the urinary excretion of histamine between attacks and probably a subnormal excretion during attacks thus suggesting an error of the metabolism of histamine in this disease (1) These two patients also had a factor in plasma increasing the capillary permeability

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Three biologically active systems thus seem to be affected in hereditary angioneurotic edema — the kallikrein kinin system the complement system and the metabolism of histamine The well documented, autosomal dominant inheritance of the disease implies that there is one single basic genetic defect Further studies on subjects with hereditary periodic edema are thus of interest not only to clarify the pathogenesis of this disease but also to investigate the normal relationship between these three systems

In 1963 Kagen and Becker (9) showed that the inhibitor of C 1 esterase also blocks kallikrein This finding thus suggested that the genetic abnormality of hereditary periodic edema was related to a deficiency in a single serum protein The recently observed marked changes of the urinary excretion of histamine suggest that there is some linkage between the kallikrein kinin system and/or the complement system on the one

The diagnosis of hypersecreting villous papilloma is often missed, even in cases where rectal palpation has been done The reason for this is that these papillomas are so soft that they cannot be felt with the finger In our case the papilloma could not be felt with certainty until its presence had been established by sigmoidoscopic examination Unfortunately, in many cases the patient's general condition is so poor that more thorough exploration of the rectum is not carried out Sigmoidoscopy with the patient lying in the left lateral position is possible, however, even in patients whose general condition is very bad If this examination had been made at an early stage in our case, the operation would probably have been performed sooner But the patient for the first 40 days was in such poor general condition that an operation would have involved great risks

Summary

Villous papillomas in the rectum and colon usually discharge mucus In rare cases this secretion may be so profuse that it results in electrolyte fluid depletion In our case the electrolyte fluid losses became excessive and led to renal failure which has not been described earlier Because of the stormy course, the patient was haemodialysed eight times and enormous amounts of electrolytes and fluid were given before the correct diagnosis was established and the tumour removed The profuse secretion from the tumour during two months permitted extensive studies of the electrolyte content and comparisons with other body fluids

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minute periods The amount of acid was determined by titration to pH 7 with 0 1 N sodium hydroxide A double lumen gastric tube was used to aspirate the gastric juice A pump with intermittent suction was used and the position of the tube was checked by fluoroscopy before the study The studies were made in the morning after an overnight fast The normal value of the laboratory as determined in healthy men was 3 7 ± 0 4 mEq/hour for the basal secretion (6) The *maximal secretion of hydrochloric acid* was determined after Histalog® stimulation (2 mg/kg body weight)

Blood samples were withdrawn and left at room temperature for 30 min After centrifugation of the tubes *serum* was immediately sucked off and stored at —60 C until analyzed *Plasma* was obtained from blood samples containing EDTA (1 5 ml 0 027 M to 20 ml blood) The samples were handled as described above for serum Before complement and esterase analyses 0 1 ml thrombin per ml plasma was added to coagulate the samples

Sensitized red sheep cells (EA) were prepared according to Pillemer et al (20)

C la (for nomenclature see ref nos 12 and 21) C la determination was based on the method of Borsos et al (3) 0 3 ml serum in double dilutions in veronal sucrose buffer pH 7 4 ionic strength 0 065 (22) was added to tubes containing one ml EA suspended in the veronal sucrose buffer The tubes were incubated at 4 C for 20 min After centrifugation the cells were washed twice in the veronal sucrose buffer and resuspended in the veronal sucrose buffer To the tubes was then added 0 25 ml MgEDTA serum as a source of C'4 C 2 and C 3 (12) Controls with veronal buffer containing MgEDTA instead of MgEDTA serum were always included in the test The degree of lysis was recorded after incubation of the tubes at 37 C for 60 min The amount of serum giving 50 % lysis was said to contain 1 unit (u) of C la *C 4 and C 2 titrations* were performed according to Pillemer et al (20)

C 1 esterase in serum and in plasma was determined using N acetyl l tyrosine-ethyl ester (ATEe, Calbiochem Luzern Switzerland) as substrate The technique described by Laurell et al (11) was used with the modification that the phosphate buffer was 0 005 M Enzyme activity was expressed in units (u) according to Levy and Lepow (18)

C 1 esterase inhibitor was determined as described by Laurell and Siboo (14) The amount of inhibitor was expressed in units (u) according to Levy and Lepow (18)

Generation of anaphylatoxin in vitro

The procedure of da Silva and Lepow (26) was used Blood was collected from the caval vein of normal male rats (Sprague Dawley weight 250—300 g) previously anesthetized with ether The blood was left to clot at room temperature for one hour The serum was separated by centrifugation at 3 500 rpm for 10 min Serum from three animals was pooled before use

0 8 ml of rat serum was preincubated at 37° C during 10 min 0 2 ml of serum or plasma from the patient was added and the incubation continued for 60 min The control samples were handled in the same manner and consisted of a) 0 8 ml of rat serum mixed with 0 2 ml of phosphate buffered saline (PBS 1 part of phosphate buffer pH 7 4 ionic strength 0 15 and 9 parts of 0 15 M NaCl) and b) 0 2 ml of the serum or plasma from the patient mixed with 0 8 ml of PBS

Assay on the guinea pig ileum

The biological activity of serum or plasma from the patient during eight different days and of anaphylatoxin activity were studied on the guinea pig s isolated ileum in a 4 5 ml bath The gut was suspended in Tyrode s solution containing atropine and glucose and maintained at 34° C with constant aeration Contractions were recorded on smoked paper on a kymograph

Only one active sample of the preparations of anaphylatoxin was assayed on each segment of the guinea pig s ileum

Mepyramine was used to block the gut contracting activity of histamine as compared to that of test samples (24)

TABLE I Laboratory and clinical data in one patient with hereditary angioneurotic edema

Day of study	Urinary histamine (µg/24 hrs)	Urinary 1 4 MeImAA (mg/24 hrs)	Gastric secretion				
			Period (mEq/30 min)				Mean value (mEq/60 min)
			1	2	3	4	
1	8	3 4	3 0	1 2	2 8	3 3	5 1
2	5	2 7					
3	10	2 8					
4	4	3 1					
5	5	3 1					
6	640	8 4					
7	34	3 9					
8	1	2 4					
9	1	3 2					
10	1	5 3	1 1	8 1	10 7	13 4	16 6
11	1	3 1					
12	9	3 0					
13	9	3 9					
14	135	8 4					
15	7	2 7					
16	7	2 4	3 0	5 2	6 2	6 4	10 4
17	4	3 6					
18	4	4 7	7 4	6 3	10 1	7 9	15 9
19	6	3 8	7 4	8 9	10 7	10 3	18 6
20	—	—	15 5	7 7	8 9	13 5	22 8
21	—	—					
22	1	2 6	3 9	2 3	6 2	4 7	8 5
23	6	2 8					
24	—	3 0	4 1	7 4	—	—	11 5
25	3	2 7					
26	4	2 4	9 0	10 0	6 5	3 0	14 5

Results

The studies on this patient were made for a period of 26 days During this time the patient had two attacks of a similar type with an interval of 14 days At the attacks which lasted for about one day and which were of only moderate severity the patient had intense nausea and epigastric pains The attacks ended with a period of vomiting No edema was observed during these two attacks Between the two attacks — eight days after the first attack — the patient noted a moderate nausea but he had no pain and no edema

At nine occasions during the study, the basal gastric secretion of hydrochloric acid was measured At the same occasions blood samples were with-

Serum complement factors			C1 esterase inhibitor		Clinical comments
C1a (u/ml)	C4 (u/ml)	C2 (u/ml)	Serum (u/ml)	Plasma (u/ml)	
					Abdominal pain Vomiting
660	<25	38	<1	<1	
					Nausea
660	<25	38	<1	<1	
990	<25	38	<1	1 24	
1980	<25	38	<1	1 32	Nausea
1980	<25	38	<1	1 48	Abdominal pain Vomiting
990	<25	38	<1	<1	
660	38	38	<1	<1	
450	75	50	<1	1 97	

drawn for the complement studies At the 24th day of the study, four days after the last attack, the maximal gastric secretion of hydrochloric acid was determined after Histalog® stimulation The following values were found 22 1, 23 3 23 3, 18 9 and 11 5 mEq HCl 30 60 90 120 and 150 min after the administration of Histalog®

The urine was sampled continuously in 24 hour periods During the last attack when the patient stayed in the hospital for two days, hydrochloric acid was by mistake omitted from the bottles in which the urine was sampled These samples could thus not be used for determination of histamine and 1 4-MeImAA in urine

TABLE I. Laboratory and clinical data in one patient with hereditary angioneurotic edema

Day of study	Urinary histamine (µg/24 hrs)	Urinary 1,4 MeImAA (mg/24 hrs)	Gastric secretion				
			Period (mEq/30 min)				Mean value (mEq/60 min)
			1	2	3	4	
1	8	3.4	3.0	1.2	2.8	3.3	5.1
2	5	2.7					
3	10	2.8					
4	4	3.1					
5	5	3.1					
6	640	8.4					
7	34	3.9					
8	1	2.4					
9	1	3.2					
10	1	5.3	1.1	8.1	10.7	13.4	16.6
11	1	3.1					
12	9	3.0					
13	9	3.9					
14	135	8.4					
15	7	2.7					
16	7	2.4	3.0	5.2	6.2	6.4	10.4
17	4	3.6					
18	4	4.7	7.4	6.3	10.1	7.9	15.9
19	6	3.8	7.4	8.9	10.7	10.3	18.6
20	—	—	15.5	7.7	8.9	13.5	22.8
21	—	—					
22	1	2.6	3.9	2.3	6.2	4.7	8.5
23	6	2.8					
24	—	3.0	4.1	7.4	—	—	11.5
25	3	2.7					
26	4	2.4	9.0	10.0	6.5	3.0	14.5

Results

The studies on this patient were made for a period of 26 days. During this time the patient had two attacks of a similar type with an interval of 14 days. At the attacks which lasted for about one day and which were of only moderate severity the patient had intense nausea and epigastric pains. The attacks ended with a period of vomiting. No edema was observed during these two attacks. Between the two attacks — eight days after the first attack — the patient noted a moderate nausea but he had no pain and no edema.

At nine occasions during the study, the basal gastric secretion of hydrochloric acid was measured. At the same occasions blood samples were with-

Urinary 1,4 MeImA 4

The knowledge of the normal limits of this substance in urine is still limited but preliminary results indicate that the upper normal limit probably does not exceed 4 mg/24 hours Markedly increased values were found at the same two days when the excretion of histamine in urine was markedly increased Low values corresponding to the low histamine values in urine were not seen after the attacks (table I)

C'1 esterase inhibitor was determined in serum as well as in defibrinated EDTA plasma No measurable amounts or very low amounts were found in all the samples (table I) The normal values of C'1 esterase inhibitor range between 16—22 u/ml

C'1 esterase in serum and defibrinated plasma was determined as ATEe hydrolyzing capacity In no instance ATEe hydrolyzing activity was found after an incubation time of 15 min However, low ATEe hydrolyzing activity was generated in serum after 30—40 min and the activity increased during the following 30—40 min up to 40—50 u/ml ATEe hydrolyzing activity was generated also in plasma on prolongation of the incubation time but a slower generation was observed compared to serum (16—24 u/ml on incubation for 70—80 min)

C'1a was determined with the immune hemolytic test (see Methods) With the technique used 50 % hemolysis was obtained with 0 3 ml of normal sera in dilution 1/100 (330 u/ml) In the serum samples from the patient higher C'1 values were found The highest value, 1,980 u/ml was found in the samples from day 19 (the day before the attack) and day 20 (during the attack) (table I) In the morning of day 19 the patient had symptoms of nausea and epigastric discomfort, but these symptoms disappeared at noon and reappeared the next morning (day 20)

C'4 and C'2 titers

Low C'4 and C'2 titers were found in the serum samples < 25 u/ml and 38 u/ml, respectively The titers for normal sera were 800—1,600 u/ml and 300 u/ml, respectively An increase was found in the C'4 titer to 38 and 75 u/ml in the samples collected the days after the attack (days 24 and 26 respectively) Also a slight increase of the C'2 titer was found on day 26 (table I)

Biological activity of plasma and serum and generation of anaphylatoxin

The studies were made on serum withdrawn on the same occasions as for the complement studies No contractions were produced by 0 1 ml of serum from the patient 0 1 ml of plasma from the same days gave a small and slow contraction after a delay of about 10 sec The contraction had no properties in common with histamine and was not blocked by mepyramine The identity of this gut contracting substance is not yet clear

0 1 ml of samples of rat serum incubated with serum or plasma (proportion 4 1) from the patient obtained during three of the eight days mentioned evoked a strong and relatively rapid response

similar to that of histamine The activity was completely abolished by mepyramine It was found, however, that incubation of a mixture of rat serum and normal human serum also resulted in formation of a substance capable of releasing histamine from the guinea pig gut No histamine releasing factor was formed on incubation of rat serum in buffer

Furthermore, investigation in the pH state showed that rat serum, in the dilution obtained with this test (final concentration of serum 1/5), contained between 90—100 u ATEe hydrolyzing enzyme per ml This activity also appeared in the same or higher strength when rat serum was mixed with normal human serum Soy bean trypsin inhibitor in a final concentration of 0 2—1 mg/ml did not inhibit the activity, nor could C'l esterase inhibitor inhibit this ATEe hydrolyzing activity No ATEe hydrolyzing activity could be demonstrated if the rat serum had been heated at 56° C for 30 min

Discussion

Marked changes of the histamine metabolism were observed in the present subject with hereditary periodic angioneurotic edema At one attack with severe gastrointestinal pain and vomiting increased amounts of histamine and 1,4 MeImAA were found in urine Unfortunately the samples were spoiled during the next attack Increased amounts of histamine and 1,4--MeImAA were also found about half way between two attacks At this time the patient had symptoms of nausea for about one day Previously the patient had also noted similar symptoms between attacks

The gastric secretion of hydrochloric acid also showed marked changes during the 26 day period of study The secretion was measured at one attack and the mean value of four periods — 22 8 mEq HCl/hour — was of a magnitude usually only seen in patients with the Zollinger Ellison s syndrome Two days after the attack an almost normal gastric secretion was observed The results thus suggest that there is a periodicity in the gastric secretion of hydrochloric acid Unfortunately, no measurements were made at the occasions when evidence of an increased histamine excretion were obtained The results show that also this parameter deserves further studies in patients with periodic edema

The present finding of an increased urinary excretion of histamine and its main metabolite, 1,4-MeImAA, demonstrates convincingly an increased formation and/or liberation of histamine during the attacks and provides a satisfactory explanation for the elevated basal gastric secretion of hydrochloric acid

In a previous study, performed in 1960 (1) seemingly contradictory findings were obtained as regards urinary histamine, since *low* values were observed *during* the attacks, whereas high values were noted between and immediately preceding the attacks The previous analyses were performed by the same method and in two different patients and the results cannot be explained by technical faults At present, no satisfactory explanation can be given for the

varying results However, six years ago the attacks in the patient were accompanied by edema whereas now only gastric symptoms were present If this is the sole explanation of the divergent results cannot be decided without further studies of the histamine metabolism during attacks of varying types Nevertheless it would seem to be established that periodic edema is associated with changes in the histamine metabolism, changes which are obviously significant for the interpretation of the pathophysiological mechanisms involved

The complement studies showed that this patient lacked the inhibitor in serum against C l esterase This finding is thus consistent with previous observations in other families with this disease (4) No C l esterase activity (as measured as ATEe hydrolyzing capacity) was found in serum or plasma when incubated for 15 min When the incubation was prolonged ATEe activity appeared indicating a generation of C l esterase which never was observed with normal serum tested under the same conditions

The low C 4 and C'2 titers in the samples collected before and during the attack may be considered as an expression of active C l esterase The inactivation of C 4 and C 2 is known to be a more sensitive test of C l esterase than the ATEe hydrolyzing activity (15) The C la values obtained with the immune hemolytic test were highest the day before and during the attack C la in lower amounts were also recorded in attack free days the lowest value was found when the C 4 and C 2 titers were the highest

EDTA has been shown to inhibit the activation of C l to C l esterase in euglobulin prepared from normal serum (14, 16) Lepow et al (17) have given support for an autocatalytic activation of C l to C l esterase The finding of a generation of ATEe hydrolyzing activity in EDTA plasma of the patient after prolonged incubation at 37° C might therefore be explained by preformed low amounts C l esterase in the plasma of the patient which catalyses the conversion of C'l to C la

Studies of da Silva and Lepow showed that when purified human C l esterase was incubated with guinea pig or rat plasma a non diffusible compound was formed which has the property to release histamine This compound was identified as anaphylatoxin The generation of this compound took place by interaction with the complement system (26)

The formation of a histamine releasing substance on incubation of the patient's serum and rat serum or plasma at first seemed to be in concordance with the findings of da Silva and Lepow (26) However, the findings of generation of a histamine releasing substance on incubation rat serum or plasma with normal human serum makes it doubtful to ascribe C l esterase in the patient s serum any role for the generation of the histamine releasing factor on incubation with rat serum

Incubation of rat serum or plasma with normal human serum or with buffer resulted in the formation of an ATEe hydrolyzing substance which was not inhibited by soy bean trypsin inhibitor or C l esterase inhibitor Further experiments to study this activity more closely were not performed

The results obtained in the present investigation may suggest that the symptoms and findings in hereditary angioneurotic edema should be explained by a liberation of histamine in the tissues It is difficult, however, to explain why antihistamines are without any effect in preventing the formation of edema in this disease However, the lack of effect of antihistamines is no proof that histamine is not involved since the release of histamine may in certain tissues result in local concentrations of histamine of such a magnitude that antihistamines cannot effectively compete

Moreover, there is never any itching which should be expected if histamine was the agent responsible for the edema of the skin The same should be valid if kinin or 5 hydroxy tryptamine were responsible The balance of evidence thus indicates that some other compound may mediate this effect on the capillary permeability in the skin The observed marked changes of the histamine metabolism, however, indicate that the control of the release or formation of histamine is deranged in this disease

As it can be presumed that there is one single basic genetic defect explaining all findings the results suggest either that the C'1 esterase inhibitor has a function also in a system controlling the release or formation of histamine or that the basic defect is situated in an earlier step leading to a defect also of other proteins than the C'1 esterase inhibitor The variation of the clinical manifestations of the disease in this patient, sometimes only edema, sometimes only gastro-intestinal disturbances and sometimes a combination of these symptoms may indicate that different systems are periodically put into action The observations that one and the same patient with this disease may at the same or at different attacks present symptoms of purpura, fever etc (23) are also consistent with such a hypothesis

It has repeatedly been pointed out by other investigators that it is difficult to explain the periodicity of the symptoms with regard to the constant lack of or the very low concentrations of C'1 esterase inhibitor Moreover, it has not been established that the C'1 esterase activity is the cause of the symptoms It may also represent a parallel running disturbance

Using the complement system as a model it is known that certain components (e g C'4 and C'2) are consumed during an attack It is sufficient that *one* component of the reaction chain is completely consumed to stop the reaction This may be the cause of the cessation of an attack A new attack can then not be initiated until *all* components of the reaction chain are resynthesized up to certain minimal levels The slowest rate of resynthesis will thus have a determining influence on the interval between attacks If the symptoms of this disease are elicited through different biologically active systems and if certain components in these reaction chains (leading to edema, abdominal pain, fever, purpura etc) are common, then it may be expected that a consumption of such a common compound in one reaction chain will also block another chain The chain which is first reconstituted will then determine not only the

interval between attacks but also the type of symptoms at the attack Such a hypothesis may be supported by the different effect on C 2 in attacks with different symptomatology as judged from data in a previous study (2) In our patient abdominal symptoms occurred at one attack when the concentration of C 4 and C 2 was low also before the attack whereas only marked edema occurred in the following attack (not described in this paper) when the concentrations of C 4 and C'2 had again started to increase

The different patterns of the urinary excretion of histamine in this patient as reported in the present and a previous study (1) may also be explained by such a hypothesis that different systems are activated

Summary

The studies were made in one patient with hereditary angioneurotic edema who had no or extremely low amount of the C 1 esterase inhibitor in serum C 1a was found in highest amount the day before and during the attack

Incubation of the patients serum or plasma with rat serum generated an anaphylatoxin like substance However, such a histamine releasing substance was also formed when incubating rat serum and normal human serum

Increased urinary excretion of histamine and its main metabolite, 1--methyl-4 imidazoleacetic acid, were observed at one attack and just between two attacks when the patient had only slight symptoms of nausea The basal gastric secretion of hydrochloric acid was repeatedly measured and was markedly increased during an attack

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The Effect of Lumbar Sympathetic Block upon the Nutritive Blood-flow Capacity in Intermittent Claudication

A metabolic study

By

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The value of lumbar sympathectomy in the treatment of intermittent claudication has been a matter for dispute as is evidenced by the extensive clinical literature (for ref see 13) The disagreement may be due partly to differences in the principles of case selection but probably mainly to the fact that the evaluation of clinical results has commonly been based on the patients subjective statements

Several attempts have indeed also been made to evaluate objectively the effect of lumbar sympathetic block or sympathectomy on the muscle blood flow in cases of intermittent claudication However, most of these investigations have dealt with resting conditions and and are thus clearly not directly relevant to the assessment of therapy in cases with symptoms of circulatory insufficiency during exercise only Sometimes the changes in muscle blood flow capacity after sympathectomy have been estimated in terms of reactive hyperemia in the calf after exercise or arterial ischemia (1, 9, 21, 23) but the results are far from uniform Walder (27) tried to assess the effect of sympathectomy on muscle blood flow during exercise by following the disappearance rate of $Na^{24}Cl$ from depositions in the gastrocnemius muscle

The aim of the present investigation was to estimate the influence of lumbar sympathetic block upon the muscle blood flow of ischemic legs during exercise utilizing a metabolic approach The energy required by working muscles is derived mainly from aerobic metabolism but to a certain extent also anaerobically, by conversion of muscle glycogen to lactate During leg exercise of comparable intensity the oxygen saturation of femoral venous blood has been found to be lower in patients with obliterative arteriosclerosis in the leg than in normals, while at the same time

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TABLE I Some clinical and laboratory data

Case no	Age (yrs)	BSA* (m²)	Walking tolerance (m)	Oscillography (calf index / forearm index)	Occl. pleth. graphy (calf) (ml/100 ml/min)	
					Rest	Peak flow
Group I						
1	41	2.04	175	0.6	4.0	9.2
2	68	1.72	325	0.7	2.9	21.7
3	54	1.70	> 400	1.1	4.3	21.8
4	67	1.70	175	0.2	3.3	9.6
5	51	1.94	225	0.5	2.1	13.4
6	46	1.86	400	0.3	3.5	13.6
7	46	1.95	> 400	0.8	3.0	17.2
8	62	1.77	250	0.7	3.1	14.6
9	54	1.96	> 400	0.9	1.4	16.2
10	59	2.05	300	0.6	2.6	10.8
Group II						
11	37	1.88	300	0.2	3.4	8.4
12	59	2.11	280	0.5	2.7	11.8
13	44	1.74	> 400	0.5	3.3	14.8
14	64	2.06	155	0.2	2.2	7.7
15	59	1.78	300	0.4	2.3	12.3
16	61	1.85	225	0.3	3.6	14.7
17	54	1.73	300	0.1	2.6	6.7
18	58	2.09	325	0.2	3.3	11.6
19	53	2.00	175	0.4	1.6	6.8
20	64	2.12	250	0.2	4.0	14.8

Arteriography	
Main arterial obstruction	Accessory obliterative arterial changes
A short segmental almost complete obstruction of a il comm sin	
A il comm sin totally occluded Well developed collaterals	The distal part of a tib.ant occluded
A short partial stenosis of a il comm.sin	
A 15 cm long occlusion of a fem.sup dx distal to a prof fem Good collaterals	A il comm moderately narrowed
A 14 cm long occlusion of a fem sup.sin distal to a prof.fem Good collaterals	
A 20 cm long occlusion of a fem.sup dx with good collaterals	
A 20 cm long occlusion of a fem.sup dx with well developed collaterals	
A short almost complete obstruction of a fem.sup sin 1 dm proximal to the knee joint	Some irregularities in the lower leg arteries
A popl dx has a 3 cm long total occlusion with a well developed collateral circ	Small irregularities in a fem.sup and the lower leg arteries
—	—
Total occlusion of a il comm dx	Severe obstructive changes in the distal part of a.fem.sup a popl and in the lower leg arteries
A il comm dx has a short severe stenosis just distal to the bifurcation	Several more or less complete obliterations of the lower leg arteries Irregularities in a fem sup
A 3—4 cm long occlusion of a fem sup dx at the adductor passage	All arteries of the legs markedly thin
The entire a fem sup and a popl dx occluded	The lower leg arteries largely replaced by irregular collaterals
A fem sup.sin has *multiple stenoses the most* severe 20 cm proximal to the knee joint	A tib ant *obliterated the other lower leg* arteries narrow
A fem.sin has a 5 cm long occlusion at the adductor passage	A il extremely tortuous a fem irregular and the lower leg arteries narrow Slow circulation
A fem.sup dx extensively occluded	Severe obliterative changes in a prof.fem a popl Slow collateral circ
A fem.sup dx shows multiple stenosing obliterations *where lumen is less than 1 mm in diam*	Less severe obliterative changes in a.popl and a fem prof
A popl dx obliterated within a long segment just above the knee joint	Marked irregularities of a fem.sup The lower leg arteries extensively thrombosed
A fem sup sin occluded distal to a prof fem	A popl markedly narrowed A tib post occluded

the femoral venous lactate concentrations were higher (7) The differences were considered to express the inability of the diseased leg to accomodate the muscle blood flow to the degree of exercise, giving rise to a compensatory increase of the regional oxygen utilization and lactate formation The dependence of *these metabolic factors upon the muscle* blood flow capacity has recently been confirmed in a similar study before and after arterial reconstructions (2) Accordingly, a comparison of the degree of oxygen utilization and lactate formation in the leg during identical periods of exercise before and after lumbar sympathetic block was expected to give information about this possibility of improving the blood flow of the leg muscles in the presence of arterial insufficiency

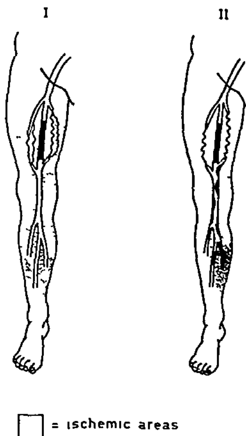


Fig 1 Schematic illustration of the group differences in the localisation of the arterial obliterations in the leg The dotted areas indicate the probable degree and extent of ischemia in the leg muscles during the maximal exercise tolerated

Material

The present series comprised 20 male patients with intermittent claudication due to obliterative arteriosclerosis in the legs The reduced walking tolerance had been present for 1—7 years with an average of 3 years In a standardized walking test four patients managed more than 400 m while the others had to stop earlier because of intolerable muscular pains in the leg (table I) The results of leg arteriographies (table I) were used to separate the cases with mainly isolated segmental occlusions of the iliac superficial femoral or popliteal artery (group I = cases no 1—10) from those with further obliterative arterial changes above all in the lower leg arteries (group II = cases no 11—20) Schematic drawings of the two types of cases are given in fig 1 In one patient (no 10) arteriography was not performed but clinical evidence and the oscillographic results suggested that he belonged to group I because of a segmental block of the right femoral artery The functional state of the peripheral circulation was evaluated by oscillography and venous occlusion plethysmography over the calves (table I)

Five of the patients (nos 3 4, 14 15, 16) had electrocardiographic changes suggestive of coronary heart disease at rest or during exercise but in no case did anginal complaints appear before the exercise test on the bicycle ergometer had to be interrupted because of ischemic pains in the leg One patient (no 18) had auricular fibrillation and moderate enlargement of the heart in spite of being

digitalized The other patients had regular sinus rhythm and no clinical signs of congestive heart failure All were normotensives and non diabetics None was on vasodilator drugs

Methods and procedure

Oscillography The arterial pulsations in the calves and forearms were registered with an oscillograph (Infraton OS 3 Bouche AB, Tubingen) After volume calibration the highest amplitude was taken as the index of the respective level

Venous occlusion plethysmography A Dohn plethysmograph as modified by Graf and Westersten (12) was used for determination of the resting blood flow of the calves as well as the peak flow values 10 sec or later after a 5 minute period of arterial occlusion over the thigh The methodological errors of oscillography and venous occlusion plethysmography have been given previously (2)

Skin temperatures were measured with thermocouples fixed to the skin with tape and connected to a direct reading mirror galvanometer (TE 3 Ellab Denmark)

Arteriography (Dept of Roentgenology at Serafimerlasarettet Stockholm) was performed without general anesthesia the contrast being administered via the abdominal aorta through percutaneous catheters into the contralateral femoral artery The grouping of the patients was checked by an experienced roentgenologist

Metabolic investigation Each patient performed a mechanically identical one leg work on a bicycle ergometer before and after a temporary lumbar sympathetic block

Blood was sampled before and intermittently during and after exercise for analysis of the oxygen content and the concentrations of lactate and pyruvate The samples were obtained from percutaneously inserted teflon catheters placed in the femoral vein and a brachial artery The oxygen uptake was determined at rest and during each working intensity and the heart rate was followed electrocardiographically For further details of the procedure and analytical methods the reader is referred to a previous paper (2)

The initial submaximal load was usually 150 kpm/min with an increase in intensity of 150 kpm/min every five minutes until the patient had to stop because of intolerable pain of the ischemic type in the leg muscles Suitable loads had been arrived at individually during exercise tests some days before the metabolic investigation Accordingly one patient was allowed to start with 75 kpm/min with a later increase to only 150 kpm/min, while another started directly with 300 kpm/min The maximal load range was 150—600 kpm/min The exercise performed before the lumbar sympathetic block was exactly repeated afterwards in each case within two hours

Five of the patients (nos 2 8 10 14 and 16) were re examined in the same way six months after injections of phenol solution around the lumbar sympathetic ganglias given to produce a long term sympathetic blocking effect

Lumbar sympathetic block The *temporary* blocks were obtained by the deposition of 15 ml 0 5 % mepivacaine (Carbocain®) at the venterolateral sides of the vertebrae L II and L IV respectively The large volumes used were presumed to secure an extensive infiltration within the paravertebral space and along the iliac vessels In two cases this was confirmed by checking the distribution of an added contrast medium (Urografin®) The efficiency of the block was judged from tests with the sympathogalvanic reflex (SGR) as provoked by pain stimulation and registered from plate electrodes on the dorsal and plantar sides of the foot (18) A satisfactory block was considered to have been achieved when deflections indicating changes in electrical skin resistance were extinguished in the blocked leg but not in the other one Because of an initially unsatisfactory response in this respect the blocking procedure had to be repeated in one case

The changes in digital skin temperature after the block were followed and the second exercise test was generally not started until

ml/min in group II (table I), the difference being probably significant ($p < 0.05$). There was no group difference concerning the resting blood flow of the calf.

At the check six months after the phenol blocks there was a tendency to improved oscillographic pulsations and peak flow values of the calves in the three patients belonging to group I, while the corresponding values were essentially unchanged in the other two patients (fig. 3).

Metabolic Investigation

A. The temporary blocks (table II, figs. 4 and 5).

Heart rate. In a sitting position just before the beginning of exercise there was a general tendency to a higher heart rate after the block. During work this difference levelled off in the patients belonging to group II while it was maintained by the group I patients.

Oxygen uptake and working intensity. Within each patient group the mean oxygen uptake was virtually the same before and after the block at rest as well as during exercise of comparable intensity. Patient group I had a somewhat higher mean oxygen uptake than group II at the highest work load, corresponding to a similar discrepancy in mean working intensity (375 ± 106 and 285 ± 34 kpm/min respectively). These differences, however, are not statistically significant. The total amount of work performed averaged 3135 ± 1830 kpm in group I compared with 1678 ± 390 kpm in group II ($p < 0.05$).

Oxygen content of blood. The mean arterial *oxygen capacity* of blood was for all patients 17.4 ± 1.7 g/100 ml before the first exercise compared with 16.6 ± 1.9 g/100 ml before the second ($p < 0.001$). At the end of exercise there was, however, no difference between the two examinations in this respect.

The *arterial oxygen saturation* averaged at rest before the block 95.0 ± 2.6 % and during exercise at the highest load 96.1 ± 2.3 %. These means for the whole material were essentially the same after the block.

The femoral *venous oxygen saturation* at supine rest was significantly increased after the sympathetic block (mean 65.1 ± 9.2 and 75.5 ± 7.4 % respectively). Although it occurred at a considerably lower level, this difference was more pronounced in the sitting position. During exercise, on the other hand, the femoral venous oxygen saturation was virtually the same at comparable loads before and after the block within the two patient groups. The oxygen saturation of blood sampled from the femoral vein just before exercise was interrupted averaged 10—15 %, without any significant group difference. The influence of the block on the femoral a—v oxygen differences corresponded to that which could be expected from the changes of the femoral venous oxygen saturation.

Lactate. The resting mean *arterial* concentration of lactate was for the whole series almost identical before and after the sympathetic block. During exercise before the block there was a successive increase to means of 3.81 ± 0.97 and 2.37 ± 0.37 mmoles/l respectively within the two pateint groups at the maximal load ($p < 0.001$). Compared with the means before the block, there

was a tendency afterwards to lower arterial lactate concentrations during exercise within group I, but the effect of the block in this respect was not significant in either group

The femoral *venous* mean concentration of lactate was also of the same order at rest before the two exercise periods The level of femoral venous lacate during exercise was significantly lower after the block than before within group I, the mean concentrations at the maximal load being 4 62 ± 1 58 and 6 31 ± 2 06 mmoles/l respectively ($p < 0.01$) A similar difference was found 3 and 5 min after exercise The group II patients, on the other hand, had mainly unchanged femoral venous lactate concentrations during exercise after the block At the end of exercise before the block the mean concentration of lactate in femoral venous blood was higher in group I than in group II ($p < 0.01$)

The *venous arterial* lactate difference at the maximal load was higher before than after the block within group I (means 2 50 ± 1 52 and 1 50 ± 1 06 mmoles/l respectively, $p < 0.01$), while in group II it was virtually the same at the end of the two exercise periods

Excess of lactate (XL) The concept of XL was introduced by Huckabee (15) as a means of compensating for the influence upon the lactate concentration in blood of pyruvate formation stimulated by factors irrelevant to tissue hypoxia This excess fraction of total lactate concentration in arterial blood was calculated according to the original formula

$$XL = (L_n - L_o) - \frac{L_o}{P_o}(P_n - P_o)$$

where L_o and P_o are the arterial concentrations of lactate and pyruvate at rest and L_n and P_n the corresponding concentrations during exercise However, like the arterial total lactate concentration, the arterial XL concentration was not significantly influenced by the block in either patient group

The regional formation of XL in the exercising limb was estimated from the differences in lactate and pyruvate concentrations between the femoral vein (v) and artery (a) and from the basal (o) arterial lactate/pyruvate ratio

$$v—a\ XL\ diff = (L_v - L_a) - \frac{L_o}{P_o}(P_v - P_a)$$

This modification of Huckabee's original formula was suggested by Hood et al (14) While these authors treated the basal L/P ratio as a constant we preferred to calculate it individually from the resting arterial concentrations at each investigation Thus obtained the mean v—a XL difference of patient group I at the highest load was 2 12 ± 1 34 mmoles/l before the block compared with 1 22 ± 0 95 mmoles/l after the block This difference is significant ($p < 0.005$) In group II the corresponding means were 0 63 ± 0 66 and 0 94 ± 0 77 mmoles/l respectively ($p > 0.05$) The mean v a XL difference of group I at the highest load before the block was significantly higher than that of group II ($p < 0.01$)

B Control investigation

A series of patients with intermittent claudication of a severity corresponding to that of the above mentioned groups I—II and with a similar age range, was studied metabolically during two

TABLE III The mean differences of some metabolic parameters between determinations from two consecutive identical periods of heavy leg exercise in the controls compared with the metabolic effects of lumbar sympathetic blocks in the patient groups I and II

			No of observ	Mean diff	SD of mean diff	P
Controls	Oxygen uptake	l/min	6	+0 02	0 06	
	Fem ven O_2 sat	%	8	—1 1	1 8	
	Fem a—v O_2 diff	ml/l	7	+1	6	
	Fem ven lact	mmoles/l	9	—0 25	0 64	
	Fem v—a lact diff	mmoles/l	9	—0 15	0 38	
	Fem v—a XL diff	mmoles/l	7	—0 01	0 25	
Patient group I	Oxygen uptake	l/min	8	—0 02	0 05	—
	Fem ven O_2 sat	%	10	+1 0	4 5	—
	Fem a—v O_2 diff	ml/l	10	—5	10	—
	Fem ven lact	mmoles/l	10	—1 69	1 18	<0 01
	Fem v—a lact diff	mmoles/l	10	—1 00	0 93	<0 05
	Fem v—a XL diff	mmoles/l	10	—0 89	0 77	<0 01
Patient group II	Oxygen uptake	l/min	7	+0 02	0 13	—
	Fem ven O_2 sat	%	10	+2 0	6 7	—
	Fem a—v O_2 diff	ml/l	10	—5	11	—
	Fem ven lact	mmoles/l	10	+0 17	0 78	—
	Fem v—a lact diff	mmoles/l	10	+0 05	0 58	—
	Fem v—a XL diff	mmoles/l	10	+0 31	0 73	—

The p values indicate the significance of the mean metabolic changes in each patient group when tested against the mean differences from the control investigation

identical one leg exercise tests without any lumbar block in the 1—2 hour interval Paired observations made just prior to the interruption of exercise were used for calculations of possible systematic errors Even allowing for these results, the metabolic effect of the block within group I was significant (table III), giving lower femoral venous concentrations of lactate at the end of a leg exercise of comparable intensity and duration after the block than before This was also true for the simultaneous changes of the femoral veno arterial concentration differences of lactate, total as well as 'excess"

C The permanent blocks with phenol

The oxygen capacity and saturation and the concentrations of lactate and pyruvate in arterial and femoral venous blood found initially in this metabolic check six months after the phenol blocks agreed well with the resting values found before the temporary blocks At the end of a leg exercise closely corresponding in intensity and duration with that performed at the investigation six months earlier, the patients belonging to group I now showed an average decrease of the a—v oxygen difference and v—a lactate difference over the exercising leg (fig 6) In the

two patients from group II these metabolic parameters were essentially unchanged as can be seen from the same figure The femoral v—a XL differences of the group I patients showed the same tendency to lower values after the phenol block as before with means of 0 64 and 1 72 mmoles/l respectively at the end of the highest load

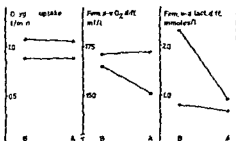


Fig 6 The mean effect of the phenol blocks upon the femoral a—v oxygen differences and the v—a lactate concentration gradients as determined at comparable rates of total oxygen uptake just before interruption of identical periods of leg exercise before (B) and six months after (A) lumbar phenol blocks for three group I patients (○—○) and two group II patients (●—●)

Discussion

The significantly higher oxygen saturation of femoral venous blood at rest after the block than before was in consistency with the plethysmographic finding of an increased peripheral blood flow The big increase in digital temperatures after the blocking procedure indicates that the supplementary blood flow was largely distributed to the skin vessels The particular effect of the sympathetic block on the oxygen saturation of femoral venous blood in the sitting position was to be expected in view of the normally high vasoconstrictor activity in the legs under orthostatic conditions (6) The progressive decrease in the femoral venous oxygen saturation during exercise — down to values below 2 % in the most extreme cases — without any differences between the saturation levels as found on the two separate occasions suggests that a sympathetic block does not markedly interfere with the normal diversion of regional blood flow towards the exercising muscles The maintenance of a high degree of oxygen extraction from blood in the exercising leg is of course not incompatible with an improved blood flow It may be the appropriate way of utilizing a moderate increase in the regional blood flow capacity

In group I the femoral venous lactate concentration and the venous arterial concentration differences of total as well as 'excess lactate, were significantly lower at the end of identical exercise periods after than before the temporary sympathetic blocks Assuming that there is an identical regional energy demand during exercise periods of comparable intensity and without signs of a compensatory increase of the oxygen extraction from the blood in the exercising leg after the block, this difference in femoral venous lactate concentration presupposes an improved leg blood flow after the block An increase of the regional blood flow would lower the venous lactate concentration levels simply by dilution However the particularly big differences between the lactate concentrations of femoral venous blood towards the end of the exercise before and after the block suggest that in addition, the formation of lactate in the active muscles

was reduced after the block This in turn is difficult to explain without an improved nutritive blood flow

Catecholamines are known to activate the phosphorylase system of skeletal muscle (8) but little is known as to their quantitative importance for the breakdown of glycogen during exercise It is, however unlikely that the decrease in lactate formation after the sympathetic block in group I was due to a withdrawal of this neurohumoral stimulation of glycogenolysis, since such a mechanism should have had a similar influence on the results in group II

The control study estimates how far the femoral venous lactate concentrations are influenced by systematic errors connected with exercise repeated at short intervals, e g effect of training and early depletion of the regional glycogen stores The good reproducibility of the metabolic variables found in the controls agreed with the results earlier obtained in healthy young men (22) From table III it is evident that it was only the patients with isolated arterial obstructions who had signs of a decreased anaerobic metabolism of the leg muscles after the sympathetic block and thus probably improved nutritional bloodflow capacity Eight of these patients also admitted that they had lesser or no muscular pains during the second period of leg exercise in spite of identity in intensity and duration In group II on the other hand, none of the patients reported a corresponding difference in complaints

From a metabolic point of view, the response to the long term sympathetic blocks with phenol revealed a group difference in agreement with that for the temporary blocks (fig 6) The tendency seen in group I, towards lower femoral a—v oxygen and v—a lactate differences during exercise at the investigation six months after the block than at comparable loads before, is accordingly suggestive of an improved nutritional blood flow in the leg Similar conclusions were drawn by Faraco and Condorelli (11), from their observations after sympathectomy of lower lactate concentrations in femoral venous blood as determined immediately after a standardized walking test and compared with the values before the operation The metabolic changes after the block in the group I patients were associated with an increase of the muscle blood flow capacity as evaluated by venous occlusion plethysmography (fig 3) and with considerable improvement of the walking tolerance

It has been shown that during exercise of high enough intensity the influence from the sympathetic vasoconstrictors on the vascular bed of skeletal muscles is rapidly overcome by the competitive effects of local metabolic factors (16) The evidence now obtained of an increased nutritive blood flow in exercising muscles after a lumbar sympathetic block may therefore seem to be incompatible with basic physiological knowledge It is not unreasonable, however, to suppose that the peripheral blood flow capacity might be promoted if there is a proximally located collateral system to be influenced

The existence of a vasoconstrictor control of collateral vessels is well

established at rest (24, 26) During exercise it is likely that the adaptation of the collateral blood flow to the increased metabolic demand is mainly passively regulated through the increased pressure head built up in particular by the dilatation of the peripheral vascular bed Thulesius (26) has further emphazised the contributory importance of the myogenic ascending vasodilating mechanism which has earlier been suggested to be active in large arteries However, experimental evidence of a persisting vasoconstrictor tone in collateral *systems even during heavy exer*cise have recently been given by Van de Berg et al (3) In dogs with an artificial stenosis of the external iliac artery these authors thus found that sympathectomy resulted in a decrease of the pressure drop across the stenosis and an impaired blood flow in the main artery during a standardized leg exercise induced by electrical stimulation In all the patients of the present study the peripheral blood flow capacity of the leg was largely dependent on the vascular resistance of a collateral system by passing the obstruction of the main artery

The difference between the two patient groups in the response to lumbar sympathetic blocks may be related to the importance of a properly functioning peripheral network of arteries for an adequate distribution and utilization within the leg of any increased blood flow brought about by a release of the vasoconstrictor influence upon the proximal collateral vessels When the bicycle exercise was interrupted because of intolerable pains in the leg muscles, the mean concentration of lactate in femoral venous blood was significantly lower in group II than in group I This difference suggests that in cases with more or less extensive obliterations of the terminal arterial branches a marked ischemia in a strictly limited part of the musculature may force the patient to stop the exercise at a point when the degree of ischemia is still moderate elsewhere in the leg muscles (fig 1) Although the grouping of some borderline cases is perhaps questionable the validity of the arteriographic *subdivision was supported* by existing group differences in arterial function as evaluated from the arterial pulsations and the peak flow values for the calves

The effect of sympathectomy upon intermittent claudication has often been regarded as being unrelated to the localisation or extent within the leg of the arterial obliterations (4, 19 20) On the other hand there also exist clinical studies indicating that an improvement is mostly seen in cases with isolated and particularly high obliterations of the main artery of the leg and where there is a well developed by passing collateral system (10 17, 25) The outcome of the present metabolic investigation further emphasizes the importance of intact arteries in the lower leg for a promoting effect of lumbar sympathetic blocking procedures upon the nutritive muscle blood flow capacity

Summary

Twenty patients with intermittent claudication were examined before and after

a temporary lumbar sympathetic block At rest and intermittently during a stepwise increasing one leg bicycle exercise, blood was sampled from catheters in the femoral vein and a brachial artery, and was analyzed for the content of oxygen, lactate and pyruvate After the block and when metabolic basality had been restored the exercise test and blood sampling were repeated exactly The stimulating effect of the sympathetic block on the muscle blood flow at rest was verified by venous occlusion plethysmography over the calves During exercise at comparable loads some of the patients had lower femoral v—a concentration differences of lactate — total as well as expressed in terms of "excess" lactate — after the block than before, in spite of a preserved level of oxygen extraction from femoral venous blood Assuming that there was an identical energy demand in the active muscles at the two periods of leg exercise, these results suggest an improved nutritive blood flow in the exercising leg after the block A comparison of the individual results with previous arteriographic examinations emphasizes the importance of well developed collateral systems, and of good functioning of the distal arteries in the leg for a favorable effect of the block upon the muscle blood flow as estimated metabolically This effect was thus found to be significant only for the part of the material comprising cases with isolated obstructions of the main artery of the leg while in patients with more extensive arterial obliterations the metabolic changes after the block did not differ from those in a control study Patients of the former type were also the only ones who later showed a positive clinical and metabolic response to permanent lumbar sympathetic blocks produced by means of phenol injections

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Immediate and Delayed Metabolic Reactions in Well-trained Subjects after Prolonged Physical Exercise

By

BJORN AHLBORG and JOHAN BROHULT

A recent study has shown that heavy prolonged standardized exercise is followed by increases in the enzymes GOT CPK LD and OCT, as well as in serum iron, in subjects with a low or ordinary physical working capacity by Swedish standards (3) The aim of the present study was to investigate the same enzyme reactions and serum iron under similar experimental conditions in physically well trained individuals

Methods

By reproducibility is meant the error of the method as calculated from duplicate determinations expressed as a percentage of the mean of these determinations

$$\frac{100\sqrt{\frac{\sum d_i}{2n}}}{\bar{x}}$$

ECG at rest and during exercise were recorded with CH leads

The *physical working capacity* was determined as the quantity of work that the subject can perform on a bicycle ergometer at a heart rate of 170 beats/min W_{170} (34) ad modum Karolinska sjukhuset

The radiological *heart volume* was determined in the supine position

Haemoglobin was determined spectrophotometrically in capillary samples by the oxyhaemoglobin method

For the following parameters venous punctures were made with disposable needles The normal values are those used at Danderyd s Hospital

GOT and *GPT* were determined by the NADH method (20 39) Normal values 10—35 Karmen units Reproducibility 4 %

CPK was determined by the method of Tanzer and Gilvarg as modified by Bernt and Bergmeyer (7) Normal value less than one nanomole (15) Reproducibility 12 %

LD was determined by the method of Wroblewski and La Due (38) Normal value 100—300 Wroblewski units One unit corresponds to a consumption of 0 53 micromoles NADH per litre and minute Reproducibility 3 %

The *OCT* activity was determined by incubation of serum with carbamoyl ^{14}C citrulline in arsenate buffer (27) Normal value less than four nanomoles which is 0 004 micromoles ^{14}CO (6) Reproducibility 7 %

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TABLE I Some anthropometric and other data in 12 subjects

Subject	Age (yrs)	Height (cm)	Weight (kg)	Heart volume (ml)	W_{170} (kpm/min)
1	21	180 0	67 8	800	1 150
2	22	176 0	69 8	890	1 300
3	21	184 5	70 4	790	1 300
4	21	178 5	68 0	970	1 200
5	23	181 0	89 7	1 120	1 550
6	22	179 0	74 8	760	1 200
7	23	181 5	82 6	900	1 500
8	21	185 0	76 5	1 030	1 650
9	21	188 0	73 7	1 030	1 100
10	21	192 0	75 6	880	1 500
11	22	178 0	73 6	900	1 400
12	22	185 5	80 7	1 010	1 300
$\bar{x}$	22	182 4	75 3	923	1 346
SD	0 8	4 7	6 5	111	174
Range	21—23	176 0—192 0	67 8—89 7	760—1 120	1 100—1 650

TABLE II Work loads work times weight loss and energy developed in 12 subjects

Subject	Mean load		Duration (min)	Weight loss		Energy developed × 10⁴ (kpm)
	Absolute (kpm/min)	% of W_{170}		Absolute (kg)	% of body weight	
1	1 035	90	94	1 6	2 4	9 7
2	1 170	90	90	1 6	2 3	10 5
3	1 050	81	96	1 5	2 1	10 1
4	1 050[1]	88	90	1 7	2 5	9 5
5	1 055[1]	68	90	1 9	2 1	9 5
6	950	79	90	1 3	1 7	8 6
7	925[1]	62	93	1 6	1 9	8 6
8	1 105[1]	67	90	1 2	1 6	10 0
9	1 250	114	90	1 3	1 8	11 3
10	1 145[1]	76	90	1 6	2 1	10 3
11	980[1]	70	90	1 3	1 8	8 8
12	965[1]	74	90	1 1	1 4	8 7
$\bar{x}$	1 057	80	91	1 5	2 0	9 6
SD	97	14	2 1	0 2	0 3	0 9
Range	925—1 250	62—114	90—96	1 1—1 9	1 4—2 5	8 6—11 3

[1] The loads have been changed once or twice

Serum iron was determined by the method of Heilmeyer and Plotner as modified by Agner (1) Reproducibility 5 %

Material

The study was made on 12 well trained young men who had just completed their first military service Certain anthropometric and other data are shown in table I

Experimental conditions

After fasting for about 12 hours the subjects were set to work as hard as possible for about 90 min on a bicycle ergometer Loads at which the subjects could exercise for about 90 min were chosen by means of pilot tests In some cases the load could be kept constant throughout the test in others it had to be changed once or twice (table II) During the exercise water was given per os ad libitum Blood samples were drawn at the beginning and end of exercise and then after 1 4 7 10 and 14 days During the sampling period the subjects refrained from physical activity and the consumption of alcohol They also abstained from alcohol for one week before the start of the test

Results

A *During exercise*

The average performance was 9 6 10^4 kpm (range 8 6 10^4—11 3 10^4), corresponding to a load of 1 057 kpm/min if the exercise had been performed at a constant load, the work time being 91 min (range 90—96) The relative load — average absolute load in relation to W_{170} — was 80 % (range 62—114) (table II) The quantity of water given per os during the exercise averaged 0 7 l (range 0 2—1 2) The absolute loss of weight during exercise averaged 1 5 kg (range 1 1—1 9) corresponding to 2 0 % (range 1 4—2 5) of the body weight prior to exercise

B *After exercise*

The *GOT* level before exercise averaged 22 Karmen u (range 17—32) and at the end of exercise 27 u (range 17—45) The difference is significant ($< 0\ 05$) The mean for day 1, 26 u (range 17—53), does not differ statistically from the initial value (table III)

The *GPT* level before exercise averaged 20 Karmen u (range 14—24) and at the end of exercise 21 u (range 11—29) This difference is not significant and neither were any of the changes in the GPT level during the observation period (table IV)

The *CPK* level before exercise averaged 0 4 nanomoles (range 0—1 1) and at the end of exercise 0 7 nanomoles (range 0—1 7) the difference being statistically significant ($p < 0\ 05$) None of the other differences obtained in this parameter during the study were significant (table V)

Total *LD* at the beginning of exercise averaged 210 Wroblewski u (range 170—245) and at the end of exercise 238 u (range 200—310) This difference is statistically significant ($p < 0\ 01$) On day 1 the mean was 249 u (range 175—450) which is a statistically significant difference from the value before exercise ($p < 0\ 05$) On day 4 the mean had returned to the initial level (table VI)

The *OCT* level before exercise averaged 1 0 nanomole (range 0 3—2 1) and at the end of exercise 1 3 nanomoles (range 0 2—4 3) The highest mean

TABLE III GOT (Karmen u) at start and the end of exercise and 1, 4 7 10 and 14 days after exercise

Subject	Start	End	1	4	7	10	14
1	32	45	34	13	14	14	—
2	27	31	29	31	23	22	19
3	18	17	20	12	17	16	16
4	20	29	24	13	20	18	18
5	20	43	20	14	15	22	17
6	17	26	53	32	32	24	22
7	17	18	29	18	24	23	22
8	20	21	20	82	31	22	21
9	23	23	17	19	21	17	21
10	25	24	19	23	21	18	26
11	19	22	26	18	24	21	23
12	20	24	17	19	22	17	20
$\bar{x}$	22	27	26	25	22	20	20
SD	4 5	8 9	10 1	19 3	5 5	3 2	2 9
Range	17—32	17—45	17—53	12—82	14—32	14—24	16—26

TABLE IV GPT (Karmen u) at start and the end of exercise and 1, 4 7, 10 and 14 days after exercise

Subject	Start	End	1	4	7	10	14
1	24	28	22	13	20	30	—
2	23	27	24	24	16	26	26
3	19	20	15	18	19	20	18
4	21	23	18	19	27	24	26
5	19	20	20	22	22	28	27
6	19	29	32	28	26	18	20
7	23	27	18	18	29	26	25
8	21	23	23	31	27	21	18
9	14	11	12	16	15	10	10
10	22	20	16	21	15	15	15
11	17	14	17	17	21	15	12
12	20	15	13	12	15	12	16
$\bar{x}$	20	21	19	20	21	20	19
SD	2 8	5 9	5 5	5 7	5 2	6 5	6 0
Range	14—24	11—29	12—32	12—31	15—29	10—30	10—27

TABLE V CPK (nanomoles) at start and the end of exercise and 1 4 and 7 days after exercise

Subject	Start	End	1	4	7
1	0 21	1 59	1 48	0 42	0 53
2	0 00	0 42	0 42	0 11	0 11
3	0 64	0 11	0 64	0 21	0 00
4	0 21	0 32	0 00	0 32	0 21
5	0 00	0 00	0 74	0 32	0 32
6	0 00	0 74	7 63	1 06	0 53
7	0 21	0 42	0 42	0 53	0 00
8	0 64	0 85	0 85	2 01	1 38
9	0 32	0 74	0 64	0 64	0 00
10	1 06	1 38	0 95	0 53	1 17
11	0 64	1 70	3 71	0 64	0 11
12	0 53	0 42	1 48	0 53	0 53
$\bar{x}$	0 37	0 72	1 58	0 61	0 41
SD	0 33	0 56	2 13	0 50	0 46
Range	0 00—1 06	0 00—1 70	0 00—7 63	0 11—2 01	0 00—1 38

TABLE VI LD (Wroblewski u) at start and the end of exercise and 1 4 7 10 and 14 days after exercise

Subject	Start	End	1	4	7	10	14
1	245	270	275	250	195	165	—
2	210	240	245	215	215	180	180
3	190	215	220	235	180	170	170
4	195	225	215	190	185	170	175
5	180	220	215	220	195	175	195
6	245	235	450	275	260	275	215
7	195	200	235	185	200	245	215
8	170	225	205	300	270	225	210
9	225	210	295	150	195	255	200
10	245	310	250	210	220	250	225
11	190	250	175	185	265	195	210
12	230	260	205	180	210	205	195
$\bar{x}$	210	238	249	216	216	209	199
SD	27	31	71	43	32	39	18
Range	170—245	200—310	175—450	150—300	180—270	165—275	170—225

TABLE VII OCT (nanomoles) at start and the end of exercise and 1 4 7 10, and 14 days after exercise

Subject	Start	End	1	4	7	10	14
1	0.9	1.5	1.5	1.2	2.1	1.6	—
2	1.2	0.7	1.4	1.0	1.7	2.5	1.6
3	1.6	1.5	1.1	0.6	1.0	0.6	0.7
4	1.0	1.7	2.3	0.9	2.8	2.6	1.3
5	0.5	1.2	0.7	2.3	1.8	3.2	1.0
6	2.1	4.3	3.0	2.6	2.5	1.7	1.3
7	0.7	0.8	0.8	0.9	1.0	1.2	0.7
8	1.5	1.4	1.5	1.8	1.9	1.1	1.1
9	0.5	0.2	0.3	1.0	0.8	0.5	0.4
10	1.0	1.5	1.1	1.5	1.6	1.0	0.8
11	0.8	0.8	1.1	0.5	0.6	0.8	1.1
12	0.3	0.3	0.2	0.1	0.4	0.7	0.4
$\bar{x}$	1.01	1.32	1.25	1.20	1.52	1.46	0.95
SD	0.52	1.06	0.79	0.73	0.76	0.88	0.38
Range	0.3—2.1	0.2—4.3	0.2—3.0	0.1—2.6	0.4—2.8	0.5—3.2	0.4—1.6

TABLE VIII Serum iron (μg %) at start and the end of exercise and 1 4 7 10 and 14 days after exercise

Subject	Start	End	1	4	7	10	14
1	136	160	148	135	96	113	—
2	114	156	164	100	187	146	97
3	120	161	142	130	141	125	114
4	128	239	168	150	162	201	151
5	103	188	125	91	93	73	121
6	108	164	168	72	90	80	122
7	90	138	102	84	70	78	130
8	113	214	141	78	68	108	149
9	138	186	186	156	192	176	162
10	78	144	214	108	136	184	143
11	120	164	150	102	118	124	158
12	146	175	156	68	80	113	104
$\bar{x}$	116	174	155	106	119	127	132
SD	20	29	29	30	44	42	22
Range	78—146	138—239	102—214	68—156	68—192	73—201	97—162

level — 1 52 nanomoles — was obtained seven days after exercise The difference between the 7 day value and the value before exercise is the only one which is statistically significant ($p < 0.05$) (table VII)

Serum iron at the start of exercise averaged 116 μg % (range 78—146) and at the end of exercise 174 μg % (range 138—239) The difference is statistically significant ($p < 0.001$) On day 1 the mean was 155 μg % (range 102—214), the difference between this and the initial value being statistically significant ($p < 0.01$) On day 4 the mean had returned to the initial level (table VIII)

Present data in relation to previous findings

The data from the present study on well trained subjects are compared below with the findings previously reported (3) for untrained subjects

The mean *age* of the present well trained individuals was 21 7 years, that of the untrained group being 20 3 years ($p < 0.05$) Their mean *heights* were 182 4 and 180 3 cm respectively ($p > 0.2$), mean *weights* 75 3 and 67 4 kg ($p < 0.05$) and *heart volumes* 923 and 787 ml respectively ($p < 0.02$) The *physical working capacity* (W_{170}) of the well trained subjects averaged 1,350 kpm/min while that of the untrained averaged 930 kpm/min ($p < 0.001$) The respective mean *loads* during the test were 1 057 and 700 kpm/min ($p < 0.001$) corresponding to 80 and 76 % of W_{170} The *working time* up to exhaustion was designed (by the choice of load) to be about 90 min for the well trained subjects, for the untrained individuals the corresponding time was about 120 min The total *amount of energy* developed by the well trained subjects averaged 9 6 10^4 kpm, that developed by the untrained group being 8 4 10^4 kpm ($p < 0.05$) Both groups had the same mean intake of water during the exercise 0 7 l The absolute weight losses occasioned by the exercise were 1 5 and 1 3 kg respectively corresponding to a relative loss of 2 0 and 1 9 % of the body weight before the exercise

The mean level of *GOT* in the well trained subjects rose from 22 to 27 Karmen u ($p < 0.05$) during the course of the exercise (table III) The corresponding rise in the untrained group — from 24 to 30 u — was also significant ($p < 0.001$) This group had an elevated mean on days 1 and 5 as well, whereas no such variation was observed in the well trained group The only significant inter group difference with respect to mean GOT occurred on day 10, when the well trained group had 20 u and the untrained 25 u ($p < 0.001$)

GPT Neither the well trained nor the untrained group displayed any significant change in mean GPT during the course of the investigation Nor were any differences observed between the groups

Mean *LD* was elevated for the well trained group at the end of exercise and on day 1 after which it displayed no significant difference from the initial

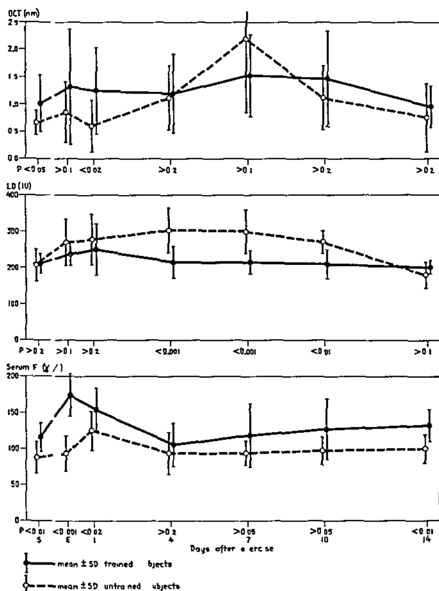


Fig 1 Variations in OCT LD and serum Fe at the start (S) at the end (E) of exercise and during 14 days thereafter

level (table VI and fig 1) The untrained group had an elevated mean from the end of the exercise until day 11 This group's mean was higher than that of the well trained group on days 4 and 7 ($p < 0.001$) as well as on day 10 ($p < 0.01$) (fig 1)

Mean *CPK* varied in the well trained group only between the start and completion of the exercise ($p < 0.05$) This group had numerically lower values than the untrained group at all times, the only statistically significant difference being on day 7 ($p < 0.05$)

Mean *OCT* was significantly elevated in the well trained group only on day 7 ($p < 0.05$) whereas this was true of the untrained group between day 4 and day 9 inclusive With the exception of day 7, the well trained group had numerically higher values than the untrained group at all times (table VII and fig 1) The inter group differences were statistically significant at the start of exercise and on day 1, the means at these times for the well trained and untrained subjects respectively being 1 01 and 0 66 nanomoles ($p < 0.05$) and 1 25 and 0 60 nanomoles ($p < 0.02$) On day 7 the untrained group s mean was 238 % of the initial value and the well trained group s 66 % The difference is statistically significant ($p < 0.01$) (fig 1)

Serum iron in the well trained group had a higher mean at the end of the exercise and on day 1 than at the start of exercise Unlike the untrained group however, the mean was not elevated after one week The well trained group had higher values throughout than the untrained group the difference being statistically significant before the exercise ($p < 0.01$), at the end of exercise ($p < 0.001$), on day 1 ($p < 0.02$) and on day 14 ($p < 0.01$) Neither group alone nor both of them combined, displayed a statistically significant correlation between the rise in serum iron on account of work on the one hand and on the other the load or the absolute amount of energy expended The correlation coefficient (r) between this rise in serum iron and the amount of energy expended is 0 34 ($p < 0.1$) for the two groups combined and 0 36 ($p < 0.2$) for the well trained subjects

Discussion

Very varied enzyme reactions have been reported in connection with physical exercise in man (12, 13, 16, 17, 19, 23, 24, 25 30 35) The present study and a previous one (3) were carried out in order to compare the effect of a heavy prolonged, standardized exercise on two groups of healthy young men, one physically well trained and the other not The comparisons made concern the effects on certain enzymes and serum iron

In such studies the readings obtained for concentrations of enzymes and other parameters are no doubt affected by the haemoconcentration This is particularly the case during submaximal, continuous exercise

It has been shown by Ekelund and Holmgren (14) that the plasma volume does in fact decrease during one hour s exercise, and this has also been reported to hold for a longer period of exercise (31) Furthermore it has been demonstrated that a major part of the decrease in plasma volume during prolonged exercise occurs during the first ten min (14 36) This suggests that one should expect a haemoconcentration of 5 to 10 % of the initial value both during brief exercise and particularly in connection with the type of exercise employed here Consequently, the slight rises of serum enzymes between the start and the end of exercise reported here for the well trained group (without correction for the blood concentration) should be

regarded as somewhat uncertain From the first day after the exercise onwards, it seems that the concentrations obtained can be assessed without having to allow for the haemoconcentration due to the exercise

GOT In view of the above mentioned concentration of blood as a result of exercise, the well trained group cannot be said to have displayed any variation in GOT during the period under review The untrained group, on the other hand, had an elevated value on day 1 after the exercise In principle, two different methods are used for analysing GOT and GPT, namely the NADH method employed by us and the method of Reitman and Frankel (29) The latter, however, is based on the principle that the keto acids released during transamination are determined by means of a dye reaction This means that pyruvic acid and ketone bodies which enter the bloodstream during exercise will produce a misleading apparent elevation of GOT and GPT Several studies using Reitman and Frankel's method of analysis have demonstrated considerable, transient rises of GOT and GPT after relatively short periods of physical exercise In view of what has been said above, these results should be treated with some caution In the present investigation, only GOT was found to be elevated after exercise The correctness of our observation that GPT was not increased in our subjects immediately and one day after exercise, is supported by the fact that we did not find any elevation of OCT in serum at these times either

CPK did not display any definite variations during the investigation, if allowance is made for changes in the plasma volume There was an elevated mean on day 1, but the difference from the initial value was not significant On this day, two of the subjects in the well trained group had a reading of more than 3 nanomoles for CPK, the corresponding number in the untrained group was 5

LD was elevated in both the well trained and the untrained group on day 1 after the exercise The mean for the well-trained subjects had returned to the initial level by day 4, whereas this took longer in the untrained group The latter thus had a higher mean LD than the former on days 4, 7, and 10

It has been demonstrated that haemolysis of the red blood cells occurs during physical exercise (22) As the red cells contain LD and as LD has a comparatively long biological half life (32), the increase in LD in the well trained group one day after the exercise might be due to a haemolytic effect The marked, sustained rise of LD in the untrained group cannot however, be explained in this way, since, judging from the serum iron, haemolysis was less pronounced in this group (fig 1)

The results for GOT, CPK and LD suggest that the well-trained group had milder after effects of the heavy exercise, as far as the muscular organs engaged are concerned Given that the well trained subjects expended a greater amount of energy than the untrained, it seems that physical training can affect

the organism in such a way as to reduce the release by cells of intracellular enzymes during strain

OCT varied within both groups, reaching a maximum in each case on the 7th day after the exercise The percentage increase from the initial value was greater for the untrained group on this day It may also be noted that the untrained group displayed the largest variations during the investigation

A high content of OCT in plasma is at present the most sensitive test of an influence on the liver (26) Since OCT is found in the liver only, the cause of its elevation after exercise must be an influence on this organ This effect on the liver could be due to the reduced circulation in this organ during physical exercise (37) This should give rise to hypoxia, which ought to have an adverse effect on the liver

On the other hand it is somewhat peculiar that an effect of hypoxia on the liver should take a week to materialize Another possibility worthy of consideration is that the stress situation as such elicited the reaction in the liver via the suprarenal glands A rise in corticosteroids and noradrenaline in the urine has been observed after the consumption of alcohol (9) and a rise in noradrenaline has also been observed after prolonged physical exercise (2) A rise in catecholamines has been noted after mental stress (21)

The reason why untrained persons show a greater affect on the liver may be that well trained individuals are more capable of maintaining the hepatic circulation and a metabolic steady state, as organisms they are better able to withstand stress

As already pointed out (3), the time of the OCT maximum in the present study agrees well with that after anaesthesia (6), burn injuries (28) and large single doses of alcohol (11) Rapid cardiac arrhythmias cardiac insufficiency and shock result in stasis of the liver and elevated values for GOT, GPT, LD and OCT in plasma (4, 5, 26, 33) These rises in enzyme levels were obtained as little as 1—5 days after the liberating mechanism had started to function However, many of the patients were observed for less than a week, so that the enzyme maximum after shock may well occur after the same interval as that following e g anaesthesia or prolonged exercise Evidence in support of this has recently been obtained in studies on anaesthetised patients and patients in shock (10)

It has been shown in vitro that intracellular enzymes such as GOT and GPT (18) can be liberated from apparently intact cells and diffuse into an ambient physiological solution When this occurs in vivo however it is held to indicate a metabolic disturbance which may or may not be injurious On the one hand it may be argued that the liberation of enzymes is a harmless physiological response to an increased load on the organism Equally however, it may be argued that the liberation of enzymes from cells whether intact or not must signify a load in excess of the organism s capacity and hence injurious in the long run The latter interpretation is strongly supported by the fact that train-

ed persons display a less pronounced enzyme increase than untrained

Serum iron was higher in the well-trained group than in the untrained on several occasions during the experiment The higher level at the start of exercise can no doubt be ascribed to the fact that the former individuals were physically fit and hence had an increased haemolysis of the red blood cells, as has been reported to occur during physical exercise (22) This circumstance probably also explains the fact that the well-trained group still had an elevated serum iron content on the first day after the exercise The only significant difference between the groups after this was on day 14, although the well trained group had higher values throughout, the lack of significance, however, was due to the large spread, a phenomenon that has already been described both at rest and during work (8) As already mentioned, the rise in serum iron directly occasioned by the exercise does show some correlation with the absolute amount of energy expended, in spite of the large spread

Summary

The serum enzymes GOT, GPT, CPK, LD and OCT are less affected in trained subjects than in untrained by a single period of prolonged physical exercise The well trained group displayed significant rises in GOT CPK, and LD at the end of the exercise as well as a significant rise in LD on the first subsequent day In the Discussion it is pointed out that these increases are partly ascribable to the haemoconcentration and haemolysis which occur during physical exercise One week after the exercise a significant increase in OCT was observed

The reasons for and significance of the elevated enzymes in conjunction with a stress on the organism are discussed Liberation of enzymes from cells appears to indicate damage to the cells

Physical training is accompanied by higher values for serum iron The well-trained group had a significant rise in serum iron both at the end of the exercise and on the next day It seems that the circumstances described should be taken into account during physical training

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Systemic Lupus Erythematosus (SLE) and Aseptic Bone Necrosis

A discussion based on the presentation of one case treated with corticosteroids

By

Ian Goldie Gösta Tibblin and Sven Scheller

Necrosis in the femoral head may appear after a fracture of the neck of the femur The traumatic etiology of this complication is obvious In other diseases a necrosis of bone may occur which is more difficult to explain, and frequently the localisation is found in the hip joints Cystic rarefaction osteosclerotic changes and destruction of the femoral head have been reported in cases of systemic disease e g sickle cell anemia (22, 24) and Gaucher s disease (8, 25) The changes have been unilateral and bilateral It has been impossible to trace any definite genetic factor and the condition has in general been regarded as aseptic necrosis

Lately Dubois and Cozen (9) have reported similar observations in systemic lupus erythematosus (SLE) They believed that a connection existed between SLE and these osseous changes Four hundred cases of SLE were investigated and eight of these had developed bilateral, and one unilateral necrosis of the femoral head Two patients had necrosis of the femoral condyle Characteristic LE cells were found in all The authors stressed that this localized bone destruction must be considered as a symptom of generalized SLE Ruderman and McCarthy (19) described a case of SLE in which six joints were affected, the hip and shoulder joints one ankle joint and one knee joint In all, 15 cases of SLE showing skeletal changes have been described by Anglo-American authors All but one of these patients had been given corticosteroids during different periods

It is well known that corticosteroids cause osteoporosis most commonly in the vertebrae, but other osseous structures may also become the site of destruction, e g the hip joints (14)

The use of corticosteroids with their known osteolytic properties may either institute facilitate or hasten the develop-

Submitted for publication January 17 1967





a b

Fig 1 Eighteen years 9 months (12/10 1962) Antero-posterior view of (a) the right and (b) the left hip-joint No pathological skeletal changes

ment of aseptic necrosis of bone in patients with SLE The appearance of osseous destructions may thus not be solely ascribed to the disease process as earlier suggested

No similar cases have been reported from Scandinavia This paper describes a patient with established SLE in whom necrosis of bone developed in both femoral heads during the course of the disease The patient was treated with corticosteroids We wish to discuss the possible correlation between the original disease, the development of osseous destructions and the use of corticoster oids

Case report

The patient is a female clerk born in 1944 who in 1960 developed a rash on her face and neck of the nettle rash type In 1961 she was treated for stomatitis Apart from this she had been well Menstruations were nor mal In July 1961 she fell ill with the follow ing symptoms face flushed and swollen fever, fatigue temporal headache recurring stomatitis, articular pain localized to knees and fingers In August the same year she was admitted to the hospital for infectious diseases from where she was referred to the Dermatological department and then to the Medical department I Sahlgren s hospital Goteborg The facial swelling and flush developed into a characteristic butterfly exanthema with scales She experienced exanthema on both legs and severe general malaise Hb 8 6/100 ml White cell count 3 200 Thrombocytes 200 000 ESR 40 mm/hr Differential count showed a relative lymphocytosis and an increased number of monocytes plasma cells maximally 4/200 On four occasions LE-cells were found The urine sediment revealed 5—15 leucocytes and bacteriuria but was later normal Serum creatinine max 1 4/100 ml Serum electrolytes ECG and roentgenograms of heart and lungs were normal Mantoux test negative for 1 mg Thymol turbidity test 0 53—0 42 units Transaminases normal No eye ground changes characteristic of SLE BMR —29 % and — 22 % respectively

Treatment

Kenacort[R], 8 mg × 3 progressively decreas ed to 4 mg × 1 Plaquenil[R] 0 2 × 3 decreasing to 0 2 × 1 The patient was discharged after three weeks One month

later Kenacort was discontinued Plaquenil somewhat later owing to loss of hair

In January 1962 she had a renewed attack with joint pain Three new tests showed LE cells Electrophoresis showed a high gamma fraction 2 2 g/100 ml otherwise normal Thymol turbidity test 0 32

Treatment

Decadron®, 0 75 mg/day at the time of discharge a week later decreased to 0 50 mg/day Her pains disappeared Prednisolone 10 mg/day Maintenance treatment Prednisolone 10 mg/day

In August 1962 symptoms recurred Now pain in both hip-joints appeared for the first time together with relapse in the joints of knees fingers ankles toes neck and lumbar region Roentgenographic examinations revealed nothing pathological except in the cervical spine where $C_V - C_{VI}$ were somewhat lower than the others Roentgenograms of the hip-joints normal (fig 1) LE-cells were found again ESR 99 mm Electrophoresis elevated γ globulins

Treatment

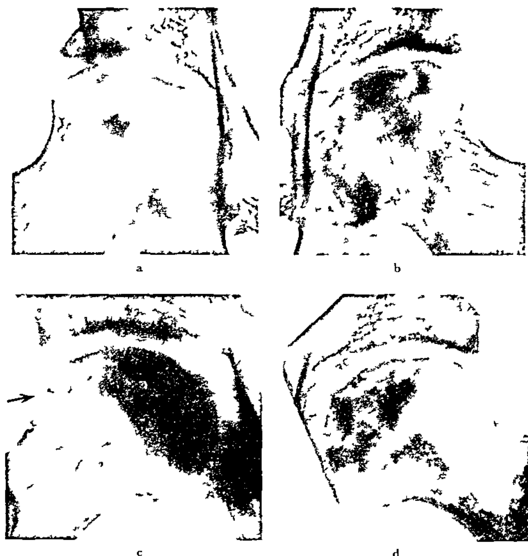
Prednisolone 5 mg × 5 for a week then 5 mg × 4 and at the end 5 mg × 2 Dianabol® 5 mg × 2 Plaquenil®, 1 × 2 Slight Cushing appearance

In March 1963 the patient fell acutely ill with abdominal pain and frequent vomiting Exploratory laparatomy showed a healthy appendix and an abundance of bright yellow peritoneal effusion Small petechiae could be seen spread in the edematous serosa of the small intestine

In April 1964 pain in the hip joints reappeared together with stiffness The patient was referred to the Orthopaedic Dept for examination Roentgenographic examination Sept 1964 of the hip-joints showed bilateral changes in the femoral head similar to those found in cases of so called aseptic necrosis of bone (fig 2) In the spongiosa there were found on one hand dissimilar, oddly shaped partly confluent rarefactions on the other patches of irregularly increased density (sclerosis) The changes in the spongiosa were most pronounced in the ventral two-thirds of the lateral half of the caput where the surface of the head was flattened in rugged and in some places defective Together with the underlying spongiosa it was depressed 3 to 4 mm In the right hip joint a bone fragment 1 cm long and 2 to 3 mm thick was displaced from the medial part of the surface of the head close by its ventral edge bordering against the collum This fragment was dislocated roughly 2 mm in a ventral direction The edge was rugged and deformed all around the head Patches of rarefaction and increased density could be found although in a lower degree in the spongiosa of the subcapital part of the collum as well as in the head The changes were somewhat more pronounced and extensive in the right joint In both hip-joints the acetabulum showed no pathological changes The patient walked with reduced movements and slightly waddling gait

Movements	Right degrees	Left degrees
Flexion	180—90	180—90
Inward rotation	20	20
Outward rotation	20	20
Abduction	30	30
Adduction	20	20

A puncture biopsy of the left hip in Nov 1964 showed at microscopy the cortex of the bone tissue is coarse and deficient in cells Areas of fibrinoid necrosis affecting both cartilage and connective tissue The bone tissue is decalcified in some places scattered centres of degeneration Between the osseous lamellae lies partly fibrinoid fat tissue No marked vascular changes No signs of specific inflammation Paget s disease or any other abnormalities of osseous tissue At the latest examination early in 1966 the patient s gait had improved Articular pains had decreased Apart from a slight cystopyelitis her general condition was satisfactory She worked full time



F g 2 Twen v eats 8 months 9 9 1964 Antero pos er or v ew of (a) the r ght and (b) the lef hip-jo nt together w th Lauens e n s projec on of (c) e r ght and (d) the left h p jo nt Changes nd cat ve of b lateral asept c necrosis rregular rarefact ons and areas of ncreased *dens ty n the head and the subcap tal par of he collum compress on of the ventral two-th rds* of the jo n surface A fragment of bone is no ced near by he ventral edge of the head of the r ght h p-jo nt deno ed by an arrow n c

Roentgenographic exami at on Dec 1964 of the hip joints sho ed progress of the previ ously demonstrated patholog cal changes of the head and femoral neck in both joints (fig 3) The rarefaction and sclerosis of the spongiosa were more extensive and the sur face of the head was further deformed owing to increased compress on n the ventral two-th rds of the head The bone fragment by the ventral edge had increased in dens ty The alterat ons were as before more ad anced in the right h p-jo nt The acetabulum showed no demonstrable changes of bone



Fig 3 Twenty years 11 months (30/12 1964) The right (a and c) and the left hip-joint (b and d) in the same projections as in fig 2 The pathological changes of the head and neck advanced since the examination three months previously (cf fig 2)

Discussion

The causes of aseptic necrosis are many A fracture of the femoral neck may finally end up in a capital necrosis There are other relevant pathological conditions some of which have been suggested above e g Sudeck s atrophy and osteolysis in cases of hemangiomatosis (12) In these cases the process is believed to depend on an increasing ingrowth of vessels causing hyperemia which disturbs the balancing powers of osteoclasts and osteoblasts This disturbance of equilibrium favors destruction which then accelerates more than production Similar mechanisms have been thought

to cause the osseous destruction in rheumatoid arthritis and in diseases of the central nervous system

Goldhaber's (11) studies on tissue cultures support these theories He found that bone cells are very sensitive to variations in oxygen saturation At a high level of saturation, 60—95 per cent, the osteoclastic resorption of bone was stimulated The effect of parathyroid hormone and vitamin D was also increased at this oxygen saturation in studies on bone in vitro

The histological sections from our patient did not show any reactive signs such as osteoclastic invasion or increased ingrowth of vessels, which may facilitate an increased oxygen saturation and thus predispose to necrosis, such as was detected on the roentgenograms and verified by microscopy The microscopic observations indicate that the biopsy was taken from a representative area Large ingrowth of vessels may, however, be limited to patches of granulation tissue occurring as single bundles of vessels, which were not observed in our sections Even if the presence of such phenomena could have been established, this would not prove anything concerning the quality or the quantity of the blood flow this determining the oxygen saturation on which the resorption of bone depends

As to pathomorphological joint changes in cases of SLE information is scanty The general idea (19, 23) is that the most important alteration appears in the vessels of the synovial tissue Acute edematous phlebitis, vascular necrosis scattered fibrinous thrombi and fibrinoid necrosis are found The term vasculitis has been used in order to obtain a general pathomorphological designation for tissue changes in SLE

In 1959 Cruickshank (7) examined tissues from 14 joints of ten cases of verified SLE He could not find indications of any of the vessel changes mentioned above In the synovial tissue an eosinophilic fibrinous substance seemed to cover the whole layer facing the joint cavity The substance contained pycnotic nuclei The synovial cells seemed decreased Further scattered, irregular hematoxylin bodies without nuclei were noticed There were no signs of inflammation

Changes of joint cartilage or bone were sparse, and those observed were small erosions with tufts of granulation tissue and deposition of fibrin Cruickshank did not consider these changes pathognomic for SLE Fibrinoid necrosis, especially if observed in other sites, might be interpreted as an indication o SLE

The cases which Dubois and Cozen (9) studied microscopically showed the same characteristics These authors emphasize, however, that fibrinoid necrosis is a pathognomic sign of SLE

The microscopic observations in the biopsy of our case conform with those of the previous three authors We have therefore been inclined to consider the necrosis of the femoral head in our patient as a sign of the generalized SLE An important complicating factor may, however, influence the development of osseous necrosis During a considerable time varying doses of corticosteroids have been administered to our patient, which may be the cause of osteoporosis

In 1965 Hastings and Macnab (14) described 125 cases of aseptic necrosis of the femoral head These cases were collected since 1950 when corticosteroid treatment was introduced In 14 cases necrosis appeared spontaneously Eight of these had been given varying doses of corticosteroids during different periods The symptoms appeared one to two years after the treatment was begun There was no case of SLE among those patients who were treated for asthma, psoriasis tendinitis hypopituitarism and iritis Pathomorphological examination of the steroid treated cases revealed no characteristic changes Great importance was ascribed to the fact that no pathological features were found in the vascular system The necrosis was therefore thought to be due to intravascular agglutination or sludging Another theory was that the blood was shunted away from the head by glomus bodies in the femoral neck This would ultimately result in aseptic necrosis The authors pointed out that steroids increase the viscosity of blood Thus intravascular agglutination would be facilitated, leading to occlusion of the end arteries with consequent avascular necrosis (2 3 15)

In 1961 Serre and Simon (20) reported ten cases of rheumatoid arthritis gout and mycosis fungoides, in which bilateral aseptic necrosis of the femoral head developed after a prolonged period of corticosteroid treatment They gave two possible explanations for the pathogenetical effect of corticosteroids firstly their destructive influence on osseous structures already brittle and subjected to repeated microtraumata secondly their influence on vessels Corticosteroid treatment, they assumed caused a great number of thrombi which also is supposed to be a feature in SLE According to these authors, such thromboses have previously been thought to be arterial or due to arteritis (17) but phlebograms indicated that they were strictly limited to veins In cases of necrosis of the femoral head an increased intra osseal pressure had been recorded This was interpreted as a sign of venous congestion due to thrombosis, especially as there was no demonstrable venous backflow such as would normally have occurred

Similar theories have been put forward by others (15 18) In 1956 Coste et al (4) proposed that the effect of corticosteroids might be vascular but were not ready to accept administration of corticosteroids as the cause of aseptic necrosis in five reported cases as these had not developed general osteoporosis On the other hand Lanyot et al (16) argued that treatment with corticosteroids certainly had caused the necrosis in 30 cases as another case with established morbus Cushing showed exactly similar changes in both femoral heads

The theory that venous thrombosis, venous stasis and decreased supply of oxygen cause aseptic necrosis of the femoral head gains some support from experimental investigations In 1964 Shaw and Bassett (21) showed that if oxygen saturation was diminished to 5 per cent osteogenesis was obstructed growth of collagen diminished and chondrogenesis reinforced

The case reported in this paper did not show any vascular changes It is quite likely that intravascular aggrega

tion, sludging and increased viscosity of blood as described by Hastings and Macnab (14) might be present Such changes may be the cause of the degeneration observed The reason for special attention to the joint disorder in this patient was the suggestion that in an acute deterioration in the patient's general condition she should be treated intensively with high doses of corticosteroids In view of the side-effects of corticosteroids engaging osseous structures, as stated above, it was thought irresponsible to subject an already damaged mesenchymal system to the hazards that the further administration of the drug may impose

This discussion demonstrates that the correlation between SLE and corticosteroid treatment of the femoral head is obscure and difficult to assess Siemsen et al s (23) words may well be applied 'The situation is quite reminiscent of the controversial note of corticosteroid treatment in the development of avascular lesions in rheumatoid arthritis

Summary

Systemic lupus erythematosus has certain features which from a pathomorphological point of view are characteristic for the disease but not necessarily pathognomonic

One of these is aseptic bone necrosis which can appear in any joint

A case history of SLE is described with advanced destructions in both hip joints, the pathogenesis of which could not certainly be ascribed to the original disease but rather to long term corticosteroid therapy

The influence of corticosteroids on osseous structures is discussed in general, and the relationship to aseptic bone necrosis in cases of SLE is commented upon in particular

A warning against heavy corticosteroid therapy in SLE is issued in view of the destructive tendencies noticed in bone

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Hyperaldosteronism Following Total Fasting in Obese Subjects

By

E Lyders Hansen Erik Hørlyck, Palle Grønbæk and Mogens Iversen

During recent years there has been a growing interest in using complete fasting as an aid to weight reduction in obese patients The method is extremely efficient and the patients endure the fasting well (1) During the management of these patients it became evident that a markedly negative fluid balance developed during fasting, that fluid was retained after termination of fasting, and that a urinary pattern of hyperaldosteronism developed evaluated on the basis of the Na/K ratio Attempts were made to counterbalance this fluid retention by administering 2 g of sodium chloride four times daily during the period of fasting and subsequently it appeared that weight reduction was then lower, that the urinary Na/K ratio did not fall below 1, and that less fluid was retained after termination of the fasting period In the aforementioned publication it was postulated that hyperaldosteronism developed in these patients, and that the fluid retention observed after cessation of fasting migh depend on this condition Recently, Rapoport et al (15) have presented some evidence that this might be the case

Bloom and Mitchell (5), Bolinger et al (8), and Schloeder and Stinebaugh (20) have shown that the weight reduction during fasting is far higher than would be expected from the consumption of calories, and they presumed this to be caused by loss of extracellular water (5) Bartter et al (2) and Duncan et al (10) have shown that changes in the extracellular volume result in an alteration in the urinary excretion of aldosterone Any enlargement of the extracellular volume will produce a decrease in the urinary excretion of aldosterone, and natriuresis will develop Such enlargement can be obtained by intravenous injection of hypotonic fluid and of vasopressin A contraction of the extracellular volume (by means of dehydration and treatment with diuretics) results in an increase in the excretion of aldosterone and in retention of sodium

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Several investigators report a large initial loss of sodium during fasting (3, 5, 8, 20) However, often the patients were studied for a few days only, and consequently the Na/K ratio in the urine was not observed over such a prolonged period as in the present study Bloom (6) showed that the loss of sodium can be prevented by glucose supplements corresponding to 600 calories per day During the period when the loss of sodium occurs, these patients receive no sodium, and sodium depletion develops It was shown clearly by Mulrow and Ganong (13) and Bledsoe et al (4) that sodium depletion results in increased production of aldosterone and increased urinary excretion of aldosterone

Material and methods

The study comprised nine patients, eight females (40 to 62 years old) and one male (55 years old) Altogether these patients were subjected to 11 periods of fasting each of ten days duration None of the patients suffered from any complicating diseases of the heart, liver, or kidneys Most of them were more than 40% overweight, according to Natvig s table (14)

The loss in weight was recorded in these patients and the Na/K ratio in 24 hour specimens of urine was determined during the ten days of fasting Apart from this exchangeable sodium and plasma volume were determined by means of ^{24}Na and ^{131}I labelled albumin, respectively (The method was described by Veall and Vetter (23)) The determinations were made on the 1st and the 8th day of fasting in some of the patients also 11 days after cessation of fasting ^{24}Na was administered orally 24 hours before determination, while ^{131}I labelled albumin was given intravenously and the blood sample for determination of plasma volume was drawn exactly 10 min after the injection Before administration of the radioactive albumin, blood samples were drawn for determination of the ^{24}Na activity This activity was also determined in a sample of the 24 hour specimen of urine Duplicate analyses were always made and the basis of calculation was that commonly applied in isotope dilution methods Between 65 and 75 microcuries of ^{24}Na were given at each test and about 5 microcuries of ^{131}I at each determination of plasma volume (maximum radiation dose 0 93 rad)

Apart from the above determinations serum sodium and serum potassium, serum protein, and haematocrit values were measured on the 1st and the 8th day of fasting and in some of the patients 11 days after termination of the fasting period Serum sodium and serum potassium values were unchanged

The determinations were made partly during complete fasting, partly when supplements of salt aldactone, or glucose were given during fasting

Results

The results are shown in table I Patients nos 5, 6 (1st period of fasting) and 8 were subjected to complete fasting Patients nos 1 (1st period of fasting), 4, 6 (2nd period of fasting) and 7 went through periods of fasting with administration of 2 g of sodium chloride four times daily Patients nos 1 (2nd period of fasting), 2 and 3 went through periods of fasting with administration of aldactone 25 mg four times daily during the fast and ten days after termination of fasting Patient no 9 received glucose, 3 5 g three times daily, during the fast

It appears from the table that all the patients lost weight during the first eight days of the fasting period In all the patients a loss of exchangeable

TABLE I Fasting periods supplements weight changes exchangeable Na, plasma volume haematocrit serum protein and Na/K ratio in 24 hour specimens of urine in nine patients

Pat no	Fasting period and supplement		Weight changes (kg)		Exchangeable Na (mEq/kg body weight)		Plasma volume (l)		Haematocrit		Serum protein (g%)		Na/K ratio in urine	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	+NaCl	+Aldactone												
	Day 1<	Day 1<	116 3	126 1	30	27	3 6	3 5	39	39	6 62	6 38	2 3	1 9
	Day 8	Day 8	111 8	119 1	24	24	3 8	3 5	40	42	6 92	7 31	1 4	0 8
	Day 21	Day 21	116 7	122 5	35	29	3 5	3 7	36	37	6 40	6 99		
2	+Aldactone													
	Day 1	Day 1	105 8		29		3 3		41		7 1		2 7	
	Day 8	Day 8	99 4		24		2 4		๖0		7 9		0	
3	+Aldactone													
	Day 1	Day 1	8๖ 8		30		2 9		37		7 1		1 6	
	Day 8	Day 8	80 7		25		2 2		51		7 6		0 4	
	Day 21	Day 21	83.2		36		3 7		42		6 9		—	
4	+NaCl													
	Day 1		99 1		30		2 4		39		6 3		3 5	
	Day 8		95 1		21		2 1		41		6 4		1 1	
5	—													
	Day 1		75 4		34		2 6		39		7 45		0 7	
	Day 8		73 0		33		2 7		39		7 01		0 5	
	Day 21		74 2		40		2 8		—		—		—	
6	—	+NaCl												
	Day 1	Day 1	118 5	110 2	32	32	3 2	3 3	35	—	—	—	0 7	1 5
	Day 8	Day 8	113 0	106 2	27	28	3 4	3 2	35	—	—	—	0 4	2 9
7	+Ṅ aCl													
	Day 1		119 6		36		3 7		41		7 0		1 9	
	Day 8		116 5		36		3 7		41		6 7		3 3	
8	—													
	Day 1		150 0		35		3 8		35		6 0		3 1	
	Day 8		139 8		26		3 1		47		7.2		0 1	
9	+glucose													
	Day 1		137 3		29		4 1		52		6 8		1.2	
	Day 8		132 1		22		3 7		51		6 7		0 6	

sodium per kg of body weight was seen, irrespective of any supplements given during fasting with the exception of patient no 7, who exhibited a weight loss corresponding only to the loss of calories during the fasting The plasma volume was nearly constant in patients nos 1, 4, 5 6 7 and 9, whereas greater

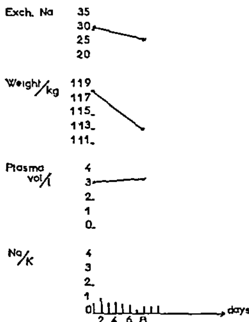


Fig 1 Exchangeable Na weight plasma volume and Na/K ratio in 24 hour specimens of urine during ten days starvation in patient no 6

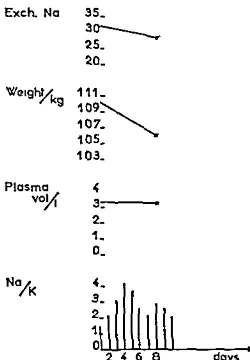


Fig 2 Exchangeable Na weight plasma volume and Na/K ratio in 24 hour specimens of urine in the same patient as in fig 1 with supplement of NaCl

variations were seen in patients nos 2, 3, and 8 The variations observed in these three patients were associated with parallel changes in the serum protein and haematocrit values

Figs 1, 2, 3, and 4 show the loss in weight, the variations in exchangeable sodium per kg of body weight and the plasma volume, as well as the Na/K ratio in the urine in patient no 6 during complete fasting and with supplement of salt, in patient no 1 who received supplements of aldactone, and in patient no 9 who was given supplement of glucose, respectively These curves are essentially identical, except that the Na/K ratio in the urine was not below 1 when sodium chloride was given during fasting From fig 3 it appears that the gain in weight after cessation of fasting coincides with an increase in exchangeable sodium even exceeding the initial value Furthermore, it appears from fig 4 that a loss of extracellular sodium cannot be prevented by glucose supplementation in the quantities stated here

Discussion

It is an established fact that during fasting the patients will exhibit a greater loss in weight than would be expected from the consumption of calories during fasting Bloom et al (5) advanced the hypothesis that this was

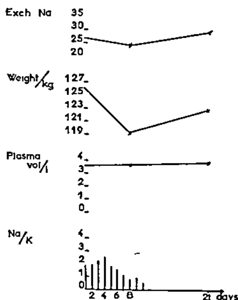


Fig 3 Exchangeable Na weight plasma volume and Na/K ratio in 24 hour specimens of urine in patient no 1 who got a supplement of aldactone

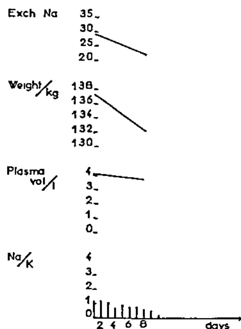


Fig 4 Exchangeable Na weight plasma volume and Na/K ratio in 24 hour specimens of urine in patient no 9 who got a supplement of glucose

caused by a loss of extracellular volume This is confirmed by our measurements of exchangeable sodium

This very limited material could not give an unequivocal picture of the normal values of exchangeable sodium per kg of body weight and the plasma volume in obese patients Furthermore, this was not the primary object, as we were interested only in measuring the changes in these values during fasting However the values obtained appear to correspond with those found by other investigators Robinson et al (17) found from 36 to 38 mEq exchangeable sodium per kg of body weight in normal males Moore et al (12) reported average values of 37 1 and 40 5 mEq exchangeable sodium per kg of body weight in normal females and males, respectively These authors found different reduced values in obese subjects

It appears from the results that a urinary pattern of hyperaldosteronism s developed as assessed on the basis of the Na/K ratio, without a concomitant decrease in plasma volume This finding was obtained in most patients From this it can be concluded that a reduction in the plasma volume is not the essential stimulus for the changes occurring in the urinary electrolyte pattern observed during fasting Recently Bloom et al (7) investigated the plasma volume in patients who fasted for varying periods (from two to six days) After a period of only two days fasting, a significant decrease in the plasma volume occurs in

all the patients The reason why our results do not agree with this finding may be that a counteracting factor (aldos terone) has exerted an influence at the time when our determinations of the plasma volume were made

The investigations described above were undertaken for the purpose of ascertaining whether hyperaldosteronism would occur in obese patients during a fasting period of ten days The results show this to be the case — A decrease occurred in the extracellular volume, measured as exchangeable sodium per kg of body weight, the patients lost sodium during fasting, no supplement of sodium was given, and these factors are pronounced stimuli for an increased production of aldosterone, which is in agreement with our finding in respect of the urinary Na/K ratio

The increased production of aldosterone may explain the retention of fluid which is observed in the patients after the end of the fasting period This is in agreement with Rapoport et al (15), who measured the aldosterone secretion rate in one patient during fasting for three weeks It appears from the results that the quantity of exchange able sodium per kg of body weight is increased after cessation of fasting, in the patients in whom this value was determined, even to a somewhat higher value than initially It appears from studies made by other researchers that perhaps this is not the sole explanation of the retention of fluid observed

Benoit et al (3) and Schloeder and Stinebaugh (21) have shown that the patients become potassium-depleted dur ing fasting, and that this depletion influences the capacity of the kidneys to acidify the urine Relman and Schwartz (16) were able to demonstrate a delayed excretion of an excess of sodium in potassium depleted patients If the patients are depleted of both sodium and potassium at the same time, a lesser increase will be obtained in the production of aldosterone than that occurring with only sodium depletion (9, 11)

Schachner et al (18) showed that an increase in the concentration of free fatty acids in the plasma occurs during fasting in obese patients Schalch and Kipnis (19) and Sussman (22) demonstrated that in both normal and obese patients showing during fasting a rise in free fatty acids in the plasma, there is a change in the glucose tolerance This might give rise to changes in the renal function which, together with the change in renal function occurring in potassium depletion, might partially explain the fluid retention which is seen in the patients after cessation of the fasting period

However, further studies into the importance of the potassium depletion and the glucose metabolism in these patients are required

Summary

Our series comprises nine patients who have been through a total of 11 periods of fasting, each of a duration of ten days During the fasting period weight changes, variations in exchangeable sodium per kg of body weight, the plasma volume, and the urinary Na/K ratio have been determined The results show

that the patients exhibit a greater loss in weight than would be expected from the consumption of calories Concurrently with the loss in weight a decrease in exchangeable sodium per kg of body weight is observed, the plasma volume remains unchanged in most patients, whereas some patients exhibit a decrease, such decrease being associated with parallel changes in serum protein and haematocrit values The urinary Na/K ratio decreased below 1 during fasting, with the exception of the patients receiving supplements of sodium chloride On the basis of the results, it is found indirectly that during ten days of fasting hyperaldosteronism will occur in obese patients This hyperaldosteronism may explain the retention of fluid observed in the patients after cessation of the fasting period

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The Urinary Sediment after Renal Transplantation

Quantitative changes as an index of the activity of the renal allograft reaction

By

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It is now possible to reverse the uremic state in patients with terminal renal disease by the use of kidney transplantation Prolonged function of the transplanted kidney is dependent upon suppression of the allograft reaction, which normally destroys a kidney transplanted between genetically different individuals of the same species

The allograft reaction is clinically reflected in an acute rejection episode a syndrome characterized by several clinical and physiological signs including fever, malaise, graft tenderness, decreasing glomerular filtration rate, oliguria and sodium retention The early detection of this syndrome may be difficult in the immediate post operative period, as several complications of diverse type may influence graft function and thereby obscure recognition The acute allograft reaction is often reversible with proper treatment and therefore it is crucial that diagnosis be established early and correctly

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Because the allograft reaction is morphologically characterized by massive infiltration of the graft by small lymphocytes and plasma cells, several investigators have undertaken serial cytologic studies of the urinary sediment on the assumption that parenchymal changes might be reflected in the cellular content of the urine Both the stained and the unstained sediment have been studied and the following have been reported as occurring with acute renal allograft rejection hematuria (7) an increase in mononuclear and epithelial cells (4) lymphocyturia (11) and the appearance of large numbers of renal tubular cells (19) Sediment examination in all the reported studies has been either strictly qualitative or only very roughly quantitative

By using a combined direct and indirect counting technique we were able to compare the daily urinary excretion rates for each type of cells found in the urinary sediment in patients after kidney transplantation

Methods

A timed urine specimen was collected daily beginning with the first day after renal allograft transplantation After a collection period of from one to three hours the urine was carefully measured in a graduated cylinder Prior to examination, which was carried out immediately after collection the urine was carefully mixed and where necessary, cleared of amorphous phosphates by a drop or two of 10 % acetic acid or of amorphous urates by gentle heating

The hourly excretion rate for erythrocytes polymorphonuclear neutrophils and renal tubular epithelial cells was determined directly using Prescott s peroxidase phloxine stain (16) One half ml of urine was transferred by pipette to a graduated tube, three drops of Prescott s stain added and the mixture brought up to the 1 0 ml mark with distilled water Both sides of a Burger-Turk counting chamber were filled with the stained urine, and at least 50 cells of each type counted Degenerate cells, squamous epithelial cells and cell debris were ignored Two counts were done and the hourly excretion rate for the various cells calculated Where cell concentration was too low to allow identification of 50 cells per 18 large squares 10 ml of urine was centrifuged at 1 800 rpm for 5 min and the supernatant discarded Three drops of stain were added to the sediment and enough distilled water to make a final volume of 1 0 ml Cell counting was as above

The hourly excretion rate for lymphocytes eosinophils basophils and macrophages was determined indirectly A smear of the sediment was made with a modification of the technique described by Strauss (18) Three drops of fresh cell free human serum were added to urine sediment obtained by centrifuging 10 ml of urine at 1 800 rpm for 5 min After careful mixing a drop of sediment was spread out very thinly on a clean microscope slide with the aid of a glass rod The slide was allowed to dry in air and then fixed for 30 sec in methyl alcohol Staining was accomplished by immersing the smear in May Grunwald stain for 15 sec washing with Nyfeldt buffer (pH 6.5), counter staining for 30 sec with Giemsa stain and washing in buffer It was occasionally necessary to double the staining times when dealing with thick smears After air-drying a permanent mount was prepared by adding a drop of balsam and a cover glass

The May Grunwald Giemsa stained smear was examined under oil immersion (1,250×) and all of the intact polymorphonuclear neutrophils lymphocytes eosinophils, basophils and macrophages identified and tabulated in each of several fields until at least ten cells of each type had been counted, giving a total cell count of from 200 to 2 000 cells Care was taken to select fields from every section of the slide according to a definite plan Duplicate determinations were done and an average taken Smears were screened before counting and discarded if staining was inadequate or the number of unidentifiable cells was greater than 10 %

Hourly excretion rates for cells identifiable only in the stained smear — lymphocytes, eosinophils basophils and macrophages — were calculated by multiplying the per-cent relationship between the numbers of one of these cells and polymorphonuclear neutrophils (as determined by examination of the stained smear) by the hourly excretion rate for polymorphonuclear neutrophils (as determined by direct counting)

Pyroninophilic cells were identified by fixing an air-dried sediment smear for one min in Carnoy s fluid staining for 10 min with Pappenheim Unna stain and examining under oil immersion

A 10 second stain, a modification of the stain described by Le Cover and Warner (13) was used to demonstrate acridine orange fluorescence

Material

Urinary cytologic studies performed in 11 patients after renal allograft transplantation are reported The kidney recipients were from 14 to 40 years of age A near relative served as kidney donor in cases nos 11, 19 20 21 and 22, a cadaver kidney was used in

TABLE I The level of excretion of urinary sediment cells on the day rejection was diagnosed

Cell excretion in 10^3 cells/hr

Case no	Rejection	Lymphocytes	Tubular epithelial cells	Polymorphonuclear neutrophils	Eosinophils	Erythrocytes
8	1st	60	240	165	0	8 000
10	1st	95	1 600	300	0	1 400
11	1st	345	710	500	25	24 500
12	1st	30	630	560	0	56 000
	2nd	180	240	250	0	510
15	1st	76	300	865	57	66 000
16	1st	360	400	3 390	300	900
	2nd	250	840	5 000	500	1 160
	3rd	75	1 120	1 520	45	240
18	1st	500	1 080	2,900	100	32 000
19	1st	125	360	5 200	60	580
	2nd	150	570	4 275	0	4 100
20	1st	30	90	40	8	540
21	1st	450	520	3,560	35	10 100

the other seven patients Only those patients whose post-operative course permitted consecutive daily sediment examination for at least one month after transplantation are included

Splenectomy and bilateral nephrectomy of the patient s own kidneys were carried out at the time of transplantation in all cases The patient s own kidneys did not, therefore contribute to the post-operative urinary sediment

Immunosuppressive therapy with azathioprine 2—4 mg/kg body weight was used post operatively in all cases and supplemented with prednisone when signs of rejection first appeared

Fourteen acute allograft rejection episodes were diagnosed on the basis of the usual criteria and without, in the first nine episodes knowledge of the urinary cytologic findings When the value of these findings became clear they were used as one of the indices of threatening graft rejection Rejection was not considered to have taken place unless there was a definite response to steroid therapy

Results

Erythrocytes, polymorphonuclear neutrophils and tubular epithelial cells

These cells were found in all urine specimens examined Their total excretion varied tremendously from 10^3 to 10^8 erythrocytes per hour, from 7×10 to 7×10^6 polymorphonuclear neutrophils per hour and from 10^4 to $2\times10^?$ tubular epithelial cells per hour There was at least a doubling in erythrocyte excretion at the time of four rejection episodes (cases nos 10, 11 20, 21) in polymorphonuclear neutrophils at the time of four (cases nos 12 16 18 21) and in tubular epithelial cells at the time of five rejection episode (cases nos 10 12, 16, 18, 21) As is shown in table I the excretion rates for the three types of cells were quite different from one rejection crisis to another Some-

times only a small number of cells were excreted, at other times a huge number were found There was no relationship between numbers of cells excreted and severity of rejection No particular level of cell excretion was characteristic of rejection, and great numbers of all three cell types could be found at times other than rejection The large, often bizarre shaped renal tubular cells mentioned by Taft and Flax (19) were observed regularly in all patients, particularly after the second post transplant week, and they did not seem to have any constant relation to rejection In cases nos 10 and 20, large tubular cells were present in great number throughout the entire post operative course

Basophils and macrophages

Macrophages were present in almost all urines studied and basophils were occasionally seen No relation was found between the urinary excretion of these cells and rejection

Eosinophils

These cells appeared or reappeared, at the time of nine of the 14 rejection crises (cases nos 11 15, 16 (3 episodes) 18, 19, 20, 21), excretion varied from 0 to 5×10^5 cells/hour (table I) Eosinophils tended to persist in the urine for one to ten days Day to day excretion varied and was without pattern, there was no relation between rejection severity and numbers excreted In only one case (no 11) were eosinophils present in the urine without there being any evidence of rejection

Lymphocytes

Two types of lymphocytes were identified in the stained sediment a small lympocyte similar to the small lymphocyte of the peripheral blood and a large lymphocyte similar to the lymphoblast of the bone marrow (fig 1) The small lymphocyte is about the size of an erythrocyte with a dense, strongly basophilic nucleus and a rim of clear, basophilic cytoplasm The large lymphocyte is about the size of a polymorphonuclear neutrophil and has a reticular, strongly basophilic round or slightly indented nucleus surrounded by a distinct nuclear membrane Nucleoli can be seen in the nucleus of some of the large lymphocytes This cell has a moderate amount of basophilic cytoplasm, which may contain basophilic granules These large lymphocytes were pyroninophilic and fluoresced yellow with acridine orange They were seen in the urine only at the time of rejection crises Clumps of lymphocytes and lymphocyte casts were not observed



Fig 1 Smear of the urinary sediment showing a large and a small lymphocyte together with two polymorphonuclear neutrophils May Grunwald Giemsa Approx × 700

Lymphocyte excretion increased sharply at the time of every clinically diagnosed rejection episode A graphical illustration of the relation between lymphocyte excretion, graft function and im

TABLE II Characteristics of lymphocyte excretion in connection with 14 acute rejection episodes in ten patients

Cell excretion in 10³ cells/hr

Case no	Rejection	Appearance significant lymphocyturia[1] (post op day)	Rejection diagnosed (post-op day)	Duration significant lymphocyturia (days)	Maximum lymphocyte excretion	Maximum lymphocyte excretion (post-op day)
8	1st	6	6	7	60	6
10	1st	3	4	8	95	4
11	1st	5	8	60	450	9
12	1st	2	2	8	350	5
	2nd	40	45	7	180	45
15	1st	1	2	16	320	1
16	1st	5	5	17	360	5
	2nd	30	30	3	250	30
	3rd	40	42	4	110	43
18	1st	5	7	11	500	7
19	1st	3	4	15	360	8
	2nd	21	30	12	200	33
20	1st	4	4	6	30	4
21	1st	4	4	9	450	4

[1] Significant lymphocyturia i e more than 25 000 cells/hr

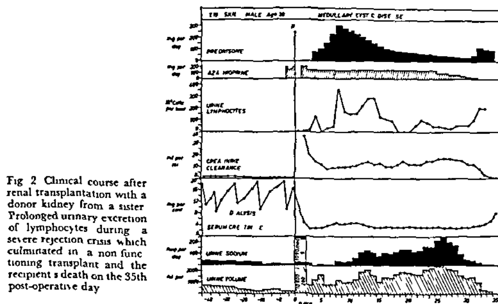


Fig 2 Clinical course after renal transplantation with a donor kidney from a sister Prolonged urinary excretion of lymphocytes during a severe rejection crisis which culminated in a non functioning transplant and the recipient's death on the 35th post-operative day

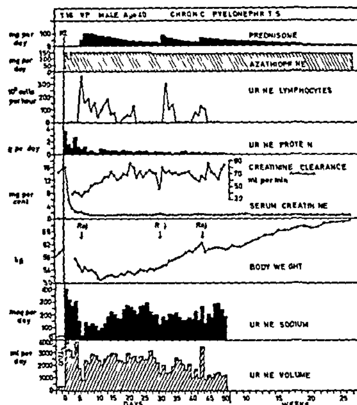


Fig 3 Clinical course after renal transplantation with a cadaver kidney Three acute rejection episodes of moderate severity each associated with the appearance of lymphocyturia occurred within the first six weeks after transplantation The recipient is well with a normally functioning transplant 12 months post transplantation

munosuppressive and prednisone therapy is given in figs 2, 3 and 4 In two patients (no 16 (third rejection) and no 21) lymphocyturia appeared before clear cut clinical evidence of rejection, making early diagnosis possible Increased lymphocyte excretion could first be demonstrated on the day when other signs of rejection appeared in the other 12 rejection episodes

Peak lympocyte excretion was usually present at the time rejection was diagnosed but in two patients (nos 12 19) it came three and four days later (table II Maximum excretion was 25,000 to 500,000 cells/hour, typically 200,000 to 300 000 The number of lymphocytes in the urine tended to decrease with the initiation of — or increase in — prednisone therapy and to reach insignificant values in the course of one to three weeks (table II)

There was no clinical evidence for graft rejection in case no 22, and lymphocyte excretion was only transiently elevated to 15,000 cells/hour on the fourth post-operative day This figure is well below that seen in association with rejection (table I)

During the second post transplant month in patient no 18, moderate numbers of lymphocytes were excreted in the urine over a two week period at the time of an acute urinary tract infection with massive pyuria and significant bacteriuria Only on this occasion was lymphocyturia greater than 25 000 cells/hour not associated with a rejection crisis

Generally there appeared to be a

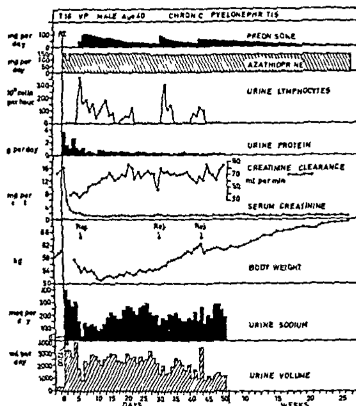


Fig 3 Clinical course after renal transplantation with a cadaver kidney Three acute rejection episodes of moderate severity, each associated with the appearance of lymphocyturia, occurred within the first six weeks after transplantation The recipient is well, with a normally functioning transplant 12 months post transplantation

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Since no technique has been described whereby the number of lymphocytes in the urine can be counted directly, it was necessary to use an indirect calculation in estimating lymphocyte excretion on the basis of the identification of polymorphonuclear neutrophils in the counting chamber and the identification of these cells and lymphocytes in May-Grunwald Giemsa stained urinary sediment smears Prescott's stain, as mentioned above provides accurate counts of polymorphonuclear neutrophils, and these cells are the easiest cells to identify in the stained sediment

The differentiation between lymphocytes and other sediment cells, particularly small renal tubular cells, is not always easy, and it can be difficult to distinguish between free nuclei and lymphocytes Strict criteria were therefore used for lymphocyte identification The only cells counted as lymphocytes were cells with a distinct, very basophilic, more-or less round nucleus and scant, basophilic cytoplasm A disadvantage of this technique is that since it is not uncommon for the total number of poly-morphonuclear neutrophils to l and the per cent lymphocytes s has a small factor cytes multiplied by a la Thus, the figures given excretion must be taken as clo mations

On the basis of this reliable sediment index of renal allograft rejection is of an excretion of more lymphocytes per hour This not a pathognomonic findi number can appear in the time of an acute urinary-tract infection with massive pyuria, as in case no 18 Lymphocyturia was not found with the other episodes of pyuria seen in the patients studied, but in these cases pyuria was nowhere near so severe and protracted Thus the finding of significant lymphocyturia (greater than 25,000 cells/hour) strongly suggests that rejection is taking place

Increased lymphocyte excretion could be demonstrated in patient no 15 in spite of threatening graft rejection taking place during the first post operative day while there was still appreciable hematuria, as the erythrocytes were counted, allowance could be made for urinary lymphocytes arising from urinary tract bleeding

Of interest is the finding of a particular cell — here called the large lymphocyte — only at the time of rejection episodes Morphologically this cell seems to [illegible] to the hemocytoblast [illegible] et al (2) as being [illegible] lymph nodes draining [illegible] rabbits as well as to the lym[illegible] cells found in cell [illegible] tur[illegible] blood after add[illegible] (3) The [illegible] ly[illegible] used in [illegible] lymphocy[illegible] up of plasma wer[illegible] lymphocytes [illegible] were [illegible] sed [illegible]

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On the basis of this study, the most reliable sediment index of threatening renal allograft rejection is the finding of an excretion of more than 25 000 lymphocytes per hour This is, however, not a pathognomonic finding as that number can appear in the urine at the time of an acute urinary tract infection with massive pyuria, as in case no 18 Lymphocyturia was not found with the other episodes of pyuria seen in the patients studied, but in these cases pyuria was nowhere near so severe and protracted Thus the finding of significant lymphocyturia (greater than 25,000 cells/hour) strongly suggests that rejection is taking place

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That eosinophils were often present in the sediment after kidney transplantation is not surprising, as these cells,

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patients detection of significant lymphocyturia (more than 25 000/hr) made early diagnosis of rejection possible.

In our hands the quantitative determination of urinary lymphocyte excretion has proved a valuable aid in the management of patients after renal allograft transplantation.

Acknowledgement

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Albumin Synthesis Rate as a Measure of Liver Function in Patients with Cirrhosis

By

ERNST HASCH, STIG JARNUM and NIELS TYGSTRUP

The serum albumin concentration is generally accepted as a reliable measure of liver function in patients with cirrhosis of the liver A low serum albumin reflects the decreased ability of the diseased liver cells to synthesize albumin (14)

A number of papers have been published on albumin turnover in human cirrhosis (1, 2, 9 14, 16) General agreement exists that the synthetic rate of albumin is low that a relatively large fraction of total albumin is often located in the extravascular space (ascites), and that the relative rate of albumin breakdown is decreased However, no systematic attempt has been made to correlate the results of albumin turnover studies with those of other liver function tests in a larger series of patients This was done in the present study, which was initiated in order to evaluate albumin turnover data as a measure of the severity of cirrhosis

Submitted for publication February 14 1967

Material

The case material comprised 21 patients with cirrhosis and 28 controls The clinical data of the patients with cirrhosis appear from table I The diagnosis was confirmed histologically in all except three (nos 3, 5 and 7) in whom tendency to bleed prevented liver biopsy The control material consisted of hospitalized patients suffering from a variety of disorders (such as disc degeneration neurosis mild arteriosclerotic heart disease) which do not influence liver function or plasma protein metabolism

Four patients with cirrhosis were studied twice (nos 2 3 7 and 13) Patients nos. 2 3 and 7 were studied just before and during prednisone treatment (about 20 mg per day) the intervals between the two studies being 3, 4 1/2 and 5 months respectively Patient no 13 was studied again after 6 months, when her condition had deteriorated considerably

Methods

Turnover studies with ^{131}I-albumin

Human serum albumin (State Serum Institute Copenhagen) was labelled with ^{131}I Iodate (15) was used for the labelling proce-

TABLE I. Clinical and laboratory data in 21 patients with cirrhosis. Nos 2, 3 and 7 were restudied

Exp no	Sex	Age (yrs)	Height (cm)	Weight (kg)	Hb (g/100 ml)	ESR (mm/h)	Serum γ-glob (g/100 ml)	Serum/choline esterase (units)	BSP retention (%)
1	[illegible]	70	16[illegible]	[illegible]0	13.5	93	3.26	33	41
2	[illegible]	71	163	60	12.3	14	1.39	94	—
2a	[illegible]	71	163	60	12.4	—	1.08	—	—
3		7[illegible]	152	[illegible]	12.7	23	3.23	137	40
3a	[illegible]	7[illegible]	152	54	11.2	14	1.15	129	—
4	[illegible]	4[illegible]	184	[illegible]	10.0	20	2.22	117	—
5	[illegible]	[illegible]	178	52	10.2	121	[illegible].11	15	26
6	[illegible]	[illegible]	172	69	10.0	57	1.31	121	41
7		72	147	60	9.6	66	3.97	81	—
7a		73	147	60	12.9	31	1.77	—	31
8		67	154	5[illegible]	7.6	100	4.67	—	29
9		71	161	61	9.6	12	1.89	—	39
10	[illegible]	7[illegible]	163	69	12.0	5	1.19	134	40
11		6[illegible]	159	71	11.4	21	1.58	—	25
12	[illegible]	70	155	[illegible]	11.1	[illegible]8	3.01	87	35
13		5[illegible]	157	53	8.0	56	5.25	91	33
13a		56	157	53	9.8	14[illegible]	[illegible].88	21	46
14		50	160	50	8.3	94	2.40	104	50
15		78	152	57	12.7	21	1.80	104	44
16	[illegible]	[illegible]9	173	75	12.4	58	1.18	—	—
17	[illegible]	[illegible]1	16[illegible]	57	14.2	40	2.92	130	38
18		[illegible]8	161	46	1[illegible].4	2[illegible]	2.04	—	—
19		[illegible]4	1[illegible]0	4[illegible]	8.7	8	1.54	67	2[illegible]
20		[illegible]	1[illegible]5	51	10.6	[illegible]	3.69	33	—
21		[illegible]3	175	89	15.1	100	1.55	—	22
Mean		[illegible]					2.67	90	3[illegible]
SD		9					1.40	38	8

* Not including repeated determinations marked with a.

dure in the first preparations, later—and in the majority of the studies—iodinemonochloride was applied (7). An average of one atom of iodine was introduced per molecule of protein. The specific activity of labelled protein was 1–2 μCi/mg albumin. Unlabelled albumin was added to prevent damage from self irradiation.

Each batch of labelled albumin was used for studies in both controls and patients with cirrhosis.

A weighed amount of ^{131}I albumin (25–50 μCi) was injected intravenously. Blood was withdrawn in heparinized tubes 10–15 minutes after the injection and at daily intervals over 8—14 days. Urine was collected in 24-hour specimens in the same period. The ^{131}I content of 3 ml aliquots was measured in a well scintillation detector.

Thyroid ^{131}I uptake was prevented by daily administration of potassium iodide (75 mg iodide twice daily).

during prednisone treatment No 13 was reexamined following a period of clinical deterioration

Alkal phosp (KA units)	Pro-thrombin (%)	S bili rubin (mg/ 100 ml)	Severely inca paci tated	GI haemor rhage	Ascites	Death liver insuff in <6 mos	Surviving > 1 y
—	22	1 4	+	+	+	+	—
10 8	54	1 5	+	—	—	—	+
—	—	—	—	—	—	—	+
13 5	70	1 8	—	—	—	—	—
—	105	0 4	+	—	—	—	—
17 4	30	2 6	—	—	—	—	+
8 2	52	1 5	+	—	—	+	—
—	80	1 5	—	+	+	—	+
20 9	51	6 3	—	+	—	—	+
—	—	0 8	—	+	—	—	—
6 3	42	1 2	+	—	+	+	—
19 0	39	2 3	+	—	+	+	—
6 1	64	1 5	—	—	—	—	+
—	—	1 1	—	+	—	—	+
6 3	—	1 6	—	—	+	—	+
40 0	90	1 6	—	—	—	+	—
7 6	15	15 5	+	—	+	+	—
16 7	59	1 8	+	+	+	—	+
34 7	64	1 5	—	—	+	—	+
10 2	59	1 6	+	—	+	+	—
11 2	33	2 8	—	—	+	+	—
44 4	48	3 8	+	+	—	—	+
24 5	40	0 6	—	—	—	—	+
10 2	46	2 6	—	—	+	—	—
21 4	86	0 9	—	—	+	—	+
16 9	54	2 0					
11 9	18	1 2					

Serum protein was determined by Kjeldahl analysis serum albumin by paper electrophoresis (6)

Albumin turnover data were calculated after the metabolic clearance method as described by Pearson et al (11)

The fractional catabolic rate (FCR) of albumin denotes the fraction of intravascular albumin (IVM) broken down per 24 hours The metabolic clearance indicates the volume of plasma which contains an amount of albumin equal to the daily breakdown Provided FCR serum albumin and body weight remain steady during the study the synthesis of albumin equals its breakdown (FCR × IVM) The distribution ratio (D) indicates intravascular albumin (IVM) as fraction of total albumin mass (TM) TM is calculated as $\frac{IVM}{D}$

Table II Albumin turnover data in 21 patients with cirrhosis compared with those of 28 control subjects (the most significant p-value in each column in italics)

		Exp no	Alb synthesis (g/24 hr)	Alb clear (ml/24 hr)	Fract catabol rate (%)	Plasma vol (ml)	Circulating alb (g)	Total alb pool (g)	Distr ratio (%)	Serum alb (g/100 ml)
		1	2.82	170	5.0	3 390	56.3	219	25.7	1.66
		2	4.01	149	4.2	3 550	95.5	239	40.0	2.69
		2a	4.21	131	4.1	3 200	102.7	224	45.9	3.21
		3	5.57	181	7.2	2 520	77.4	201	38.5	3.07
		3a	6.13	199	10.5	1 895	58.4	165	35.2	3.08
		4	9.51	362	7.5	4 820	126.8	307	41.3	2.63
		5	1.52	150	4.7	3 200	32.3	78	41.2	1.01
		6	8.67	320	8.6	3 720	100.8	244	41.2	2.71
		7	2.70	110	4.2	2 750	65.7	156	49.0	2.39
		7a	4.85	174	8.2	2 120	59.9	121	47.7	2.73
		8	4.93	233	7.5	3 100	65.7	209	31.3	2.12
		9	4.30	145	3.9	3 715	110.3	324	34.0	2.97
		10	8.87	344	9.4	3 660	94.4	229	41.1	2.58
		11	9.74	255	6.8	3 750	143.3	303	46.4	3.82
		12	6.02	216	6.9	3 130	87.3	213	40.9	2.79
		13	7.96	346	10.0	3 460	79.6	155	51.3	2.30
		13a	4.42	299	7.3	4 090	60.5	115	52.4	1.48
		14	6.93	279	8.1	3 450	85.6	147	58.0	2.48
		15	4.07	136	5.4	2 520	75.4	309	24.4	2.99
		16	12.66	307	8.2	3 740	154.4	714	21.6	4.13
		17	4.65	180	5.1	3 530	91.1	210	43.2	2.58
		18	4.12	150	6.0	2 495	68.6	179	38.2	2.75
		19	4.61	181	8.1	2 240	56.9	110	51.3	2.54
		20	5.38	209	6.9	3 070	77.9	199	39.3	2.58
		21	11.20	418	10.5	3 930	154.4	463	35.4	3.89
Cirrhotics	Mean		6.44	231	6.9	3 321	90.5	247	39.4	2.70
(n = 21)	SD		3.55	*89*	*1.9*	*602*	*32.3*	134	8.9	0.69
	Var coeff		55	38	28	23	36	54	23	26
Controls	Mean		12.38	279	9.6	3 070	130.0	308	42.0	4.31
(n = 28)	Var coeff		20	19	16	16	19	17	10	13
p of difference			0.001	—	0.001	—	*< 0.001*	*< 0.001*	—	0.001
Per kg body weight										
Cirrhotics	Mean		0.104	3.8	—	56	1.43	3.97	—	—
(n = 21)	Var coeff		40	32	—	14	24	40	—	—
Controls	Mean		0.185	4.3	—	46	1.96	4.66	—	—
(n = 28)	Var coeff		15	12	—	13	19	18	—	—
p of difference			*0.001*	—		*0.001*	< 0.001	—		
Per metre body size										
Cirrhotics	Mean		3.93	141	—	2 010	55.5	131	—	—
(n = 21)	Var coeff		51	36	—	14	33	50		
Controls	Mean		7.23	169	—	1 765	76.0	180	—	—
(n = 28)	Var coeff		17	17	—	14	18	17	—	—
p of difference			0.001	*< 0.02*		0.001	< 0.001	—		

Synthetic rate of albumin and pool masses were related to body weight and height When ascites and (or) oedema were present the estimated fluid retention was subtracted from the measured body weight

Other liver function tests

Commonly used liver function tests were applied in all patients serum bilirubin (5) (normal range 0 4—1 0 mg/100 ml) prothrombin (10) (70—130 %) alkaline phosphatases in serum (3—13 King Armstrong units per 100 ml), bromsulphalein retention (less than 7 % in the plasma 45 min after intravenous injection) and serum cholinesterase (3) (130—300 units per ml)

Clinical grading

The patients were considered as severely incapacitated if they required continuous nursing assistance because of poor general condition

Confirmed haematemesis and (or) melaena were listed as gastrointestinal haemorrhage whether the patient required transfusion or not Occult bleeding (positive benzidine reaction) was not recorded

Only unequivocal signs of ascites were accepted

The patients who died from progressive liver insufficiency within six months after the study all died in hospital Patients who died from acute gastrointestinal haemorrhage or diseases not directly related to cirrhosis were not recorded as belonging to the short survival group This also applied to living patients who had been followed for less than one year, or who died from liver insufficiency later than 6 months after the albumin study (table I)

Results

The albumin data are given in table II which also shows a comparison between patients with cirrhosis and control subjects regarding the absolute values as well as the values in relation to body weight and height The difference between the means of several parameters is highly significant ($p < 0\,001$) The highest t value (difference/standard error of difference) is that of serum albumin concentration ($t = 9\,00$), closely followed by that of the synthetic rate per kg body weight ($t = 8\,22$) The t-values of the synthetic rate in absolute terms and relative to body height are a little lower (7 09 and 7 00, respectively) Albumin clearance and plasma volume are significantly different in the two groups only when related to body weight and height, whereas total albumin mass is significantly different only in absolute terms

In three patients (nos 2,3 and 7) a repeated study performed during prednisone treatment showed that albumin synthesis rate had increased in all of them Their clinical condition had also improved Serum albumin increased in two (nos 3 and 7)

Case no 13 was reexamined following a period of pronounced progression of the cirrhosis Both serum albumin, FCR and albumin synthesis rate had decreased considerably, being only 56 to 73 % of the values observed during the first study

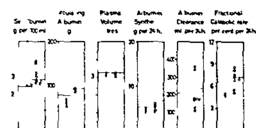
Table III shows the correlation between albumin turnover data and the conventional liver tests Several albumin data show the expected high correlation with each other, but with few exceptions the correlation between turnover data and conventional liver tests and between the individual liver tests is low

The difference between some albumin data in two clinical groups (incapacitation and survival) is shown in figs 1

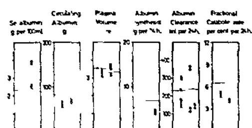
TABLE III Correlat on matr x for album n turnover data and l er funct on tests in 21 pat ents

	Alb synthesis	Alb clear	Fract catabol rate	Plasma vol	Circulat ing alb	Total alb pool
Album n synthes s		***	***	*	***	***
Album n clearance	0 84		***	***	**	—
Fract catabol rate	0 68	0 6		—	—	—
Plasma volume	0 47	0 63	—0 01		**	*
Circulat ng alb	0 8?	0 55	0 19	0 6?		***
Total alb pool	0 69	0 37	0 12	0 41	0 8?	
D str but on rat o	—0 10	0 14	0 15	0 04	—0 20	—0 6?
Serum album n	0 67	0 22	0 26	0 01	0 79	0 2
Gamma globul n	—0 39	—0 01	—0 13	0 11	—0 5?	—0 45
Chol nesterase	0 6?	0 ?4	0 38	—0 06	0 63	0 52
Pro hromb n	0 45	0 ?7	0 61	—0 3?	0 18	0 13
BSP retent on	—0 30	—0 08	—0 ?1	0 14	—0 19	—0 13
S b l n b n	—0 ?2	0 04	—0 18	0 ?6	—0 19	—0 21
Alkal phosphat	—0 01	—0 10	0 07	—0 34	—0 06	—0 06
ESR	—0 03	0 2?	0 00	0 ?8	—0 20	—0 1?

S gn f cance of correlat on coefficients *** $p < 0\ 001$ ** $p < 0\ 01$ * — $p < 0\ 05$



F g 1 Album n metabol sm in relat on to cl n cal se er y in ?1 pa ents with c rrhos s x se erely incapacitated o — moderatel n capac tated Twenty fi e t rnover stud es ere performed (see text)



F g Album n metabol sm in relat on to survi al in ?1 pa ents i h c rrhosis x death less than 6 months af er the turno er study o st ll al e one ear af er the turno er stud

and 2 Statistically no difference was present on the 5 % level The clinical grouping produced s gnificant differ ences in only three respects The mean serum gammaglobulin concentration and cholinesterase values were s gnificantly more abnormal in patients surviving less than three months (4 02 versus 1 92 g/100 ml and 58 versus 101 units per ml respectively) and the erythrocyte sedi mentation rate was significantly higher in patients with ascites (71 versus 31 mm/ hour)

with cirrhosis

Distr ratio	Serum alb	γ globulin	Cholin esterase	Pro-thrombin	BSP-retention	S bili rubin	Alkal phosphat	ESR
—	***	—	**	*	—	—	—	—
—	—	—	—	—	—	—	—	—
—	—	—	—	**	—	—	—	—
—	—	—	—	—	—	—	—	—
—	***	**	**	—	—	—	—	—
***	***	*	*	—	—	—	—	—
	—	—	—	—	—	—	—	—
—0 27		***	***	*	—	—	—	*
0 22	—0 75		**	—	—	**	—	***
—0 05	0 80	—0 68		*	—	—	—	**
—0 05	0 48	—0 35	0 53		—	*	—	—
0 02	—0 23	0 05	0 28	—0 22		—	—	—
0 28	—0 39	0 59	—0 41	—0 49	0 41		—	*
0 02	0 17	—0 16	0 16	0 37	—0 05	—0 11		—
0 09	—0 49	0 72	—0 71	—0 24	0 05	0 47	—0 32	

— = p > 0 05

Discussion

Among the albumin data examined the albumin synthesis rate showed the most pronounced reduction in patients with cirrhosis as compared with control subjects (about 50 %, table I) The serum albumin concentration was only depressed to 61 % of the normal mean The difference is statistically more pronounced, however, because the scatter is smaller

The albumin clearance and fractional catabolic rate were slightly depressed It is reasonable to regard this as a compensatory phenomenon secondary to the decreased synthetic rate In this respect our results agree with those of Wilkinson and Mendenhall (16)

From table II it appears that only the plasma volume was higher than normal in cirrhosis The increase was most pronounced when related to body weight (22 % above normal mean)

All albumin turnover data showed lower mean values in cirrhosis than in controls On the whole, the coefficients of variation in both controls and cirrhotics were lowest when albumin turnover data were related to body weight A better correlation to height might have been expected because several cirrhotic patients had ascites and oedema The difference between means of the two groups was essentially the same no matter whether absolute values or values relative to body weight or height were used

Quantitative assessment of the reduction in the physiological liver function

in patients with liver diseases is a major clinical problem which remains to be solved A priori, determination of the albumin synthesis rate seems a promising approach to answer this question, since the liver is the only site of albumin synthesis However, albumin synthesis is not necessarily a sensitive measure of decreased liver function, because the liver cells have some reserve capacity for albumin formation Thus the normal liver may increase its albumin production up to 100 %, when serum albumin is depressed due to external protein loss (4) On the other hand, the synthetic rate of albumin might be a quantitative measure when serum albumin concentration is low, because the rather small reserve capacity should then be fully engaged

In the present study albumin synthesis rate did not show a consistent relationship with either the clinical criteria or the conventional liver tests The only exception was the observation that the albumin synthesis rate rose during prednisone treatment in three patients

Several explanations of the failing correlation should be considered In the first place technical fallacies in turnover studies might obscure the results, e g a non steady state condition or inaccurate sampling of urine The quantitative role played by these factors in the present study cannot be assessed exactly, but they can hardly contribute significantly to the observed standard deviations

Another explanation might be that albumin synthesis rate in cirrhosis does not depend on the functional capacity of the liver cells, a possibility which cannot be discussed exhaustively, since we do not know in detail how the albumin synthesis is regulated under normal conditions, and still less in the cirrhotic patient It is likely that specific factors such as inadequate supply of some amino acids may limit albumin synthesis below the metabolic capacity of the liver cells This could account for the low synthesis rate in patients who appear to have a reasonably good liver function as judged from clinical criteria (e g case no 7)

It seems more difficult, however, to explain the surprisingly high synthesis rate in patients nos 16 and 21 Patient no 16 died from acute renal shut down after he had suffered from therapy-resistant ascites for 6 months It may be questioned whether the clinical classification in this case and other similar patients adequately reflects the liver function In patient no 21 the cirrhosis was discovered accidentally during surgery for bleeding peptic ulcer He might be a case of fully compensated cirrhosis, possibly even in a phase with predominant regeneration due to abstinence from alcohol during his stay in hospital A patient with a similar high synthesis rate was studied by Schwartz and Jarnum (13) during a period with general improvement, and an equally high value was found by a different technique (^{14}C carbonate) by Rosenoer et al (12) in a patient with hepatoma complicating cirrhosis

It is possible that great oscillations occur in the albumin synthesis rate depending on the changing balance between necrosis and regeneration in the cirrhotic liver If so, the great scatter of

synthesis rates reflects a very important clinical aspect of cirrhosis which has previously been practically undetectable

The new ^{14}C-bicarbonate method permits the calculation of synthetic rate of all liver synthesized plasma proteins over a period of a few hours (8) It is probable that future results with this method will reveal the pattern of protein synthesis in the cirrhotic liver

The present study with radioiodinated albumin and, in fact, most other studies concerning the clinical evaluation of liver function, shows that the basic problem is that a generally accepted, reliable reference value of hepatic function is still lacking, and that conventional liver tests and common clinical criteria are insufficient to predict the course and prognosis in the individual cirrhotic patient

Summary

Twenty-one patients with cirrhosis of the liver and 28 control subjects were studied with ^{131}I labelled human albumin Turnover data of the two groups were compared, and in the group of cirrhosis the data were compared with the results of common liver function tests and the clinical severity of the disease

A highly significant difference existed between several parameters of albumin turnover in the two groups The difference was marked in respect to albumin synthesis rate and serum albumin concentration. The relative albumin breakdown (fractional catabolic rate) in cirrhotic patients was normal or low

Within the group of cirrhosis, the statistical treatment of the data obtained showed that albumin synthesis rate was poorly and insignificantly related to other liver function tests

The reason for this discrepancy is discussed, and it is concluded that albumin synthesis rate as determined by radioiodine labelled albumin is an unreliable measure of the severity of cirrhosis and, like most other liver function tests uncertain as a prognostic guide in the individual patient

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4 Whether, in this situation, glucose and fructose are equally effective in bringing about a resynthesis of glycogen

Standardized infusions of glucose and fructose, respectively were given to healthy experimental subjects Some of them had rested before the infusion, and others had emptied their muscle glycogen store locally in one leg by hard work In some experiments, a study was made in connexion with fructose infusion of the splanchnic metabolism of glucose, fructose and lactic acid by means of hepatic vein catheterization In one experiment, the renal metabolism of these substances was also studied by means of renal vein catheterization and determination of the metabolites in the artery and renal vein blood and the urine

Material and experimental conditions

The case material consisted of healthy subjects, aged 17—48 years All experiments were started in the morning after the subjects had fasted overnight A common feature of all experiments was that muscle biopsy was performed both under basal conditions and after an infusion of glucose or fructose During the infusion, the subject was recumbent Blood samples for sugar determinations were taken repeatedly before during and after an infusion either through an indwelling arterial catheter or by pricking a finger tip During the infusion period the urine was collected for sugar determinations The subjects were divided into the following groups

Group I (9 subjects) An infusion of 20 % glucose was given, corresponding to about 4 g/kg body weight, in the course of about 4 hours Biopsy specimens were taken from one quadriceps femoris muscle before the infusion, and from the other approximately 30 min after ending it

Group II (10 subjects) A corresponding infusion of fructose was given i e 4 g/kg body weight during about 4 hours Muscle biopsy specimens were taken as in group I

Group III (8 subjects) The experiment was started by working with one leg on a bicycle ergometer (5 20) After complete exhaustion through repeated hard work biopsy specimens were taken from the quadriceps femoris muscle of the leg that had worked as well as from that of the resting leg This was followed by an infusion of glucose (4 g/kg body weight during about 4 hours) Biopsy specimens were taken from the quadriceps femoris muscle of both legs after 2 hours infusion as well as 30 min after ending it, the latter two specimens being taken successively more proximal in the muscle

Group IV (8 subjects) They were subjected to the same procedure as that in group III except that fructose was infused (4 g/kg body weight) instead of glucose

The *control group* consisted of 10 healthy subjects, who underwent muscle biopsy after an overnight fast (12 hours) as well as after a further 6 hours but who were not given an infusion of glucose or fructose In this group there was no significant difference in muscle glycogen concentration between specimens taken after 12 and 18 hours' fasting An account of this series has been given elsewhere (19)

In control experiments in healthy, resting experimental subjects the glycogen content was also determined in proximal and distal specimens of the quadriceps femoris muscle of both legs No significant difference in glycogen concentration was observed between specimens taken proximally and distally, nor between specimens from the right and left leg (19)

In 4 subjects the hepatic vein was catheterized and determinations made of the estimated splanchnic blood flow (ESBF) splanchnic glucose production splanchnic fructose uptake and splanchnic lactate balance before and during infusion of fructose Two of these subjects (Å F and E J), who were given 4 g

of fructose/kg body weight in the course of 182 and 250 min respectively (1 32 and 0 96 g/min), are also included in the aforementioned group II In the other two subjects (M H and M R), fructose was infused more rapidly during a shorter period (2 63 and 2 98 g/min during 83 and 89 min respectively) No muscle glycogen determinations were made in these 2 subjects

In one subject who underwent hepatic vein catheterization the renal vein was catheterized concurrently The PAH extraction and renal blood flow were then determined as well as the renal arterio-venous difference in glucose and fructose The urinary excretion of fructose was also determined

Methods

A detailed account has previously been given of the technique for taking the muscle biopsy, weighing the samples and determining TCA-extractable glycogen (2, 19) Glucose in blood was determined by the *o* toluidine method (18) When fructose was given, the glucose determination was made as follows 0 1 ml of blood was precipitated with 3 % trichloracetic acid 0 2 ml of the supernatant was added to 3 ml of glacial acetic acid containing 6 % redistilled *o*-toluidine and 0 2 % thiourea The mixture was placed in a water bath at 70 for 20 min and there after chilled in ice-cold water and read in a photometer at 625 mμ By this procedure no colour was obtained from the fructose Standard solutions containing 50—200 mg of fructose/100 ml gave no colour and glucose standards (50—200 mg/100 ml) with and without fructose (200 mg/100 ml) were always analysed together with the samples No difference in light absorbancy between fructose-containing and fructose free glucose standards was obtained The standard error of the glucose determination was 1 65 mg/100 ml for a single determination and 1 17 mg/100 ml for duplicate determinations

Fructose in blood was determined by the meta aminophenol method (14) The method was modified as follows 0 1 ml of whole blood was pipetted into 1 ml of phosphate buffer pH 7 4 containing 1 % glucose oxidase crude (Sigma Chemical Co) After 2 hours at room temperature or 45 min in a 37 water bath the sample was centrifuged To 0 2 ml of the supernatant was added 3 ml of glacial acetic acid containing 2 % meta aminophenol (recrystallized substance from AB Ferrosan Malmo, Sweden) After heating for 15 min in a boiling water bath the colour formed was read off in a photometer at 540 mμ A fructose solution similarly treated was used as standard The error of the method from duplicate determinations was 0 67 mg/100 ml

Lactic acid was determined enzymically with lactic acid dehydrogenase, using Boehringer s reagent kit

Bradley s technique (8) as modified by Castenfors et al (10) was used for hepatic vein catheterization and determination of the ESBF Indocyanine green was used as indicator substance

The renal blood flow was determined with PAH according to methods described earlier (3)

Results

A Standardized infusion of glucose or fructose (4 g/kg body weight)

Group I (tables I and III A) Glucose infusion after rest (9 subjects) The blood sugar rose during the infusion to hyperglycaemic values The highest glucose concentration was reached after 60 minutes' infusion (mean 380 mg/100 ml) The level then fell successively towards the end of infusion (table I) After the end of the infusion, hypoglycaemic blood sugar values were recorded in most cases During the infusion the average excretion of glucose was 21 9 g The net uptake of glucose, i e the quantity administered minus that

TABLE I Blood glucose concentration (mg/100 ml) in connexion with glucose infusion without and with preceding exercise

	Before	15 min	30 min	60 min	90 min	120 min	150 min	180 min	240 min	30 min after
Group I No exercise before infusion										
n	9	6	6	9	6	9	6	9	5	6
Mean	89.4	262.0	324.5	379.8	342.0	296.3	251.2	210.7	225.6	63.5
SE	2.2	30.4	19.0	27.4	33.8	18.3	22.3	15.5	10.7	5.8
Group III One leg exercise before infusion										
n	8			8		6		8		
Mean	92.1			279.5[1]		250.2		197.7		
SE	2.8			31.3		92.3		17.8		

[1] Almost significant difference between group I and group III ($p < 0.05$)

TABLE II Blood glucose and fructose (mg/100 ml) concentrations in connexion with fructose infusion without and with preceding exercise

	Before	15 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	30 min after
A. Blood glucose											
Group II No exercise before infusion											
n	10	10	10	10	10	10	10	10	8	6	8
Mean	85.5	99.1	105.7	111.4	109.2	102.2	100.2	97.4	90.4	92.2	80.7
S E	1.4	2.6	5.1	6.7	6.5	7.0	8.6	6.9	6.3	9.1	2.4
Group IV One leg exercise before infusion											
n	7		5		6		5		8		
Mean	90.5		110.6		103.5		101.6		103.5		
S E	2.9		2.8		2.8		3.0		3.4		
B Blood fructose											
Group II No exercise before infusion											
n		9	9	9	9	9	9	9	7	6	8
Mean		74.0	71.0	75.8	79.1	82.4	84.6	82.1	89.1	87.8	22.0
S E		12.7	10.5	6.9	6.0	6.9	9.2	6.9	6.0	7.0	4.4
Group IV One leg exercise before infusion											
n			6		6		5		7		4
Mean			77.2		73.7		95.8		85.6		28.8
S E			6.6		10.6		11.4		3.7		

TABLE III Muscle glycogen synthesis in connexion with glucose and fructose infusion No exercise before the test

Subject Sex		Age	B W (kg)	Ht (cm)	Glucose (g) Infused	Excreted	Net uptake (g/kg B W)	Glycogen content (g/100 g wet muscle tissue) Before	After
A Group I Glucose infusion									
B N	♂	19	70	187	280	25 9	3 6	1 35	1 67
K L	♀	20	56	162	200	25 7	3 1	1 21	1 77
L J	♂	20	70	178	280	15 2	3 8	1 38	1 83
L O	♂	27	70	175	300	17 6	4 0	1 62	2 00
B H	♂	23	52	174	200	33 5	3 2	1 41	1 43
L L	♂	30	92	190	370	28 7	3 7	1 45	1 75
L Z	♂	27	69	175	270	13 6	3 7	1 45	1 87
R N	♂	23	68	186	275	18 9	3 8	1 44	1 71
L K	♂	20	80	190	320	18 2	3 8	1 20	1 60
Mean						21 9	3 63	1 39	1 74
S E						2 26	0 099	0 043	0 055
Difference glycogen after—before infusion 0 35 ± 0 05 p < 0 001									
B Group II Fructose infusion									
R W	♂	26	66	165	280	7 0	4 1	1 65	2 16
M W	♂	24	62	172	250	11 6	3 8	1 03	1 38
P T	♂	27	64	174	256	13 2	3 8	1 25	1 55
A A	♂	38	72	168	288	9 7	3 9	1 60	1 79
K L	♀	20	58	162	230	12 0	3 8	1 37	1 75
B W	♀	19	52	163	210	13 3	3 8	1 06	1 47
Å F	♀	24	59	171	240	16 3	3 8	1 55	1 85
E J	♀	22	60	160	240	16 0	3 7	1 15	1 45
T E	♂	25	70	180	280	12 6	3 8	1 61	1 73
B E	♂	27	60	173	240	16 1	3 7	1 41	1 87
Mean						12 8	3 82	1 37	1 70
S E						0 94	0 036	0 074	0 076
Difference glycogen after—before infusion 0 33 ± 0 04 p < 0 001									

excreted averaged 3 6 g/kg body weight The mean rise in muscle glycogen concentration during infusion was 0 35 g/100 g muscle (table III A) This rise was highly significant (p < 0 001)

Group II (tables II and III B) Fructose infusion after rest (10 subjects) During infusion, the mean fructose concentration in blood was 71—89 mg/100 ml (table II) with a tendency to higher values during the last 2 hours The mean concentration 30 minutes after the end of infusion was 22 mg/100 ml, i e, about one fourth of that during infusion

A slight rise in blood glucose occurred in most cases during infusion It was most marked in subjects Å F and E J, both of whom underwent hepatic vein catheterization In cases R W and K L,

TABLE IV Muscle glycogen synthesis in connexion with glucose and fructose infusion One leg exercise before the test

Subject	Sex	Age	BW (kg)	Ht (cm)	Glucose (g) In fused	Glucose (g) Ex creted	Net uptake (g/kg BW)	Glycogen content (g/100 g wet muscle tissue) Non worked leg Before	Non worked leg 2 hrs	Non worked leg after	Worked leg Before	Worked leg 2 hrs	Worked leg after
A Group III Glucose infusion													
B E	♂	22	70	185	280	13 9	3 8	1 47	1 33	1 62	0 08	0 97	1 44
K E	♂	17	68	185	272	13 2	3 8	1 09	1 20	1 18	0 08	1 04	1 55
M H	♂	19	67	175	266	4 9	3 9	1 64	1 66	1 96	0 09	1 08	1 86
R H	♂	25	63	184	252	15 3	3 8	1 42	1 70	1 94	0 11	1 07	2 17
R M	♂	31	80	178	320	11 4	3 9	1 20	1 28	1 37	0 09	0 87	1 45
H M	♂	20	83	197	332	11 2	3 9	1 47	1 56	1 56	0 28	1 13	1 41
S W	♂	21	71	184	284	3 4	4 0	1 46	1 69	1 42	0 06	1 29	1 45
L O	♂	29	76	174	304	13 8	3 8	1 23	1 34	1 45	0 33	0 89	1 19
Mean						10 9	3 86	1 37	1 47	1 56	0 14	1 04	1 57
S E						1 55	0 026	0 064	0 072	0 096	0 037	0 048	0 092
B Group IV Fructose infusion													
N B	♀	44	52	162	208	19 8	3 6	1 15	1 10	1 09	0 13	0 36	0 56
M T	♀	48	57	165	228	10 8	3 8	0 75	0 90	1 04	0 04	0 40	0 70
A S	♂	27	67	177	268	12 4	3 8	1 63	1 67	1 77	0 06	0 74	1 20
J D	♂	22	81	187	324	11 1	3 9	1 72	1 74	1 82	0 31	0 74	0 86
T B	♂	23	80	176	320	14 7	3 8	1 72	1 58	1 62	0 05	0 49	0 99
J E	♂	28	65	167	260	14 3	3 8	1 44	1 68	1 89	0 04	0 73	0 91
R S	♂	24	58	183	230	11 0	3 8	1 27	1 38	1 58	0 07	0 42	0 80
A K	♂	21	62	173	248	8 7	3 9	0 86	—	1 35	0 04	—	1 07
Mean						12 9	3 80	1 32	1 44	1 52	0 09	0 55	0 89
S E						1 21	0 033	0 134	0 123	0 115	0 033	0 066	0 072

the maximum rise was inappreciable (< 9 mg/100 ml), and in case A H no rise in blood glucose was recorded

The mean urinary excretion of fructose during infusion was 12 8 g (table III B) A comparison between the excretion of glucose during glucose infusion (group I) and that of fructose during fructose infusion showed the latter to be significantly lower (p < 0 01) Glucose was demonstrable in the urine of only one subject given an infusion of fructose (B W) The mean net uptake of fructose was 3 8 g/kg body weight

During infusion, the muscle glycogen concentration rose by a mean 0 33 g/100 g muscle, i e , about the same as in infusion of glucose The rise was significant (p < 0 001)

Group III (tables I and IV A) Glucose infusion after work with one leg (8

subjects) The mean glucose concentration in blood during infusion was slightly lower than in group I in which the subjects had rested beforehand (table I) After 60 minutes, the concentration was 280 mg/100 ml, and then fell progressively towards the end of infusion The difference in blood glucose concentration between groups I and III was almost significant ($p < 0\ 05$) 60 minutes after starting the infusion

The urinary excretion of glucose during infusion was a mean 10 9 g (table IV A) This excretion was significantly lower than that in group I ($p < 0\ 01$)

The mean net uptake of glucose was 3 9 g/kg body weight The muscle glycogen concentration in the leg that had worked rose from 0 14 g/100 g muscle to 1 04 g after 2 hours infusion and to 1 57 g after 4 hours The rise was significantly greater during the early part of infusion than during the later part ($p < 0\ 01$) In the leg that had not worked, the glycogen concentration rose by 0 19 g/100 g muscle ($p < 0\ 05$) This rise was lower than that in group I, in which the subjects had rested before the infusion but the difference was not significant

Group II (tables II and IV B) Fructose infusion after work with one leg (8 subjects) Both the rise in blood fructose concentration and the urinary excretion of fructose were of the same order of magnitude as in group II (fructose infusion without preceding work) Nor did the blood glucose concentration differ significantly from that in group II

The net fructose uptake was a mean 3 8 g/kg body weight

The muscle glycogen content of the leg that had worked rose from 0 09 g/100 g muscle to 0 55 g after 2 hours' infusion, and to 0 89 g after 4 hours The rise during the first 2 hours was not significantly greater than that during the later part of infusion

In the leg that had not worked, the glycogen concentration rose from 1 32 to 1 52 g/100 g muscle ($p < 0\ 05$) Although the rise was less than that in group II, in which no work was performed before the infusion, the difference was not significant

A comparison between groups IV and III showed that the rise in muscle glycogen in the worked leg was significantly higher in group III ($p < 0\ 001$) This demonstrates that the resynthesis of glycogen after work takes place more effectively when glucose is infused than when the corresponding amount of fructose is infused

B Hepatic vein catheterization in connexion with fructose infusion (table V)

In 2 subjects (Å F and E J) 240 g of fructose were infused, and in the other 2 (M H and M R) 147 and 176 g, respectively (The results of one experiment (E J) are given in fig 1)

The ESBF increased in the two subjects (M H and M R) who were given a rapid infusion of fructose during a shorter period than the others Immediately after the end of infusion, the ESBF returned to the initial level In both subjects given a slower infusion during a longer period the ESBF

TABLE V Estimated splanchnic blood flow (ESBF) and metabolism before during and after infusion of 20 % fructose solution Results in 4 normal subjects

Subject Sex	Age	BW (kg)	Ht (cm)	Period	Time (min)	Infusion rate (g fructose min)	ESBF (ml/min)	Splanchnic Glucose production (mg/min)	Splanchnic Fructose uptake (mg/min)	Splanchnic Lactate production (mg/min)
M H ♂	22	70	187	Basal	20	—	1,510	487	—	—40
				Fruct inf	56	2 6	2 140	287	1 227	126
				After	27	—	1 580	67	292	—32
M R ♀	22	51	170	Basal	20	—	1,490	290	—	—21
				Fruct inf	59	3 0	2 520	198	1 795	306
				After	30	—	1,410	202	637	159
Å F ♀	24	59	171	Basal	23	—	2,400	324	—	—23
				Fruct inf	250	1 0	2 100	8 1	390	244
E J ♀	22	60	160	Basal	69	—	931	54	—	—15
				Fruct inf	182	1 3	1,025	20	389	138

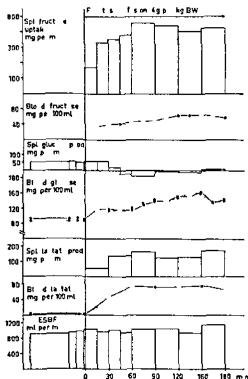


Fig 1 Estimated splanchnic blood flow (ESBF) and splanchnic metabolism before and during infusion of 20 % fructose to subject E J — — — arterial blood ——— liver vein blood

remained almost unchanged In every case, an uptake of fructose took place in the splanchnic region The splanchnic glucose production decreased considerably during infusion of fructose In all these experiments, the blood glucose concentration rose progressively during the infusion The highest blood sugar value (166 mg/100 ml) was recorded in subject E J after 2 1/2 hours' infusion In subjects E J and Å F, a negative V—A difference over the splanchnic region was recorded during the later part of fructose infusion, indicating that glucose production had ceased, and that glucose was taken up instead (fig 1)

The V—A difference in lactic acid was negative under basal conditions, which implies that a small amount of lactate was taken up in the splanchnic region (15—40 mg/min) During fructose infusion, the splanchnic production of

lactate was considerable (126—244 mg/min.). In subject Å.F. given altogether 240 g of fructose, 61 g of lactate were produced during infusion, in the other cases the production was smaller (11—25 g).

C. Renal vein catheterization

This was performed in only one subject (E.J.) who also underwent hepatic vein catheterization.

During infusion of fructose, 250 mg/min of fructose was taken up by the kidney and 88 mg/min was excreted in the urine. During the later part of infusion, the Rv—A difference in glucose was positive, indicating that the kidney produced glucose (34 mg/min.). The PAH extraction and renal blood flow were not definitely influenced by infusion of fructose.

Discussion

To permit the glycogen synthesis in muscle after infusion of fructose to be compared with that after glucose, the two sugars were given in the same quantity per kg of body weight. The net uptake — i.e., the quantity administered minus that excreted in the urine — was slightly lower after infusion of glucose than after that of fructose in the subjects who had rested before the infusion. The mean excretion of glucose during glucose infusion was twice as high as that of fructose during fructose infusion. Similar results have been reported earlier (32, 37). The difference in net uptake between glucose and fructose was however so small that it can scarcely be of any importance when comparing the two sugars with respect to the formation of muscle glycogen. The results demonstrate that, in healthy subjects who have not performed hard muscular work, the increase in muscle glycogen is about the same after infusion of glucose as after that of fructose (4 g/kg body weight).

It is not possible by studying the glycogen synthesis alone to determine whether administered fructose is taken up in the muscle directly, or whether it is wholly or partly converted to glucose in the liver or elsewhere and this, in turn, is taken up in the muscles and forms glycogen.

It is known from experiments on rat diaphragm in vitro that fructose can be taken up from the medium and incorporated into glycogen and that this effect is stimulated by insulin (17, 23, 27, 36). However, the fructose uptake is much slower than the uptake of glucose, and if both sugars are present in the medium together, the fructose uptake is partly inhibited (17, 23, 27). Studies of the peripheral arteriovenous fructose difference in connexion with fructose infusion in both the dog and man indicate that there is a peripheral uptake of fructose (22, 38, 39). In rabbits, the peripheral uptake of infused fructose was only 5 % while 80—90 % was taken up by the liver (28). Nor do experiments on eviscerated animals suggest that fructose is metabolized in peripheral tissue to any great extent (6, 40). Similar experiments with contrary results have nevertheless been presented (16).

Muscle biopsy specimens taken before and after fructose infusion in three patients in connexion with operation did not show any definite change in muscle

glycogen (38) Such results are, however, difficult to evaluate, because of the effect of the operative trauma

The rise in blood glucose obtained on infusion of fructose has been stated to be a sign of conversion of fructose to glucose (39) Also in most of our experiments, a moderate rise in blood glucose was recorded during infusion of fructose

For further investigation of how infused fructose is metabolized, hepatic vein catheterization was performed in four subjects, and the uptake and output of glucose, fructose and lactic acid from the splanchnic region were determined during fructose infusion The results show both that the liver takes up considerable quantities of fructose, and that the glucose output from the liver does, in fact, diminish during infusion of fructose This effect was particularly apparent in two experiments in which the infusion proceeded for several hours, during short periods the glucose output fell to zero, or even became negative

Earlier studies with hepatic vein catheterization in man have also demonstrated that fructose is taken up to a considerable extent in the liver (12, 25, 34, 38) Craig et al (12) found, in 2 of 3 healthy subjects, a decrease in the splanchnic output of glucose during infusion of fructose Tygstrup et al (34) did not obtain any consistent change in the splanchnic glucose production during infusion of fructose (1 1—1 8 g/min for 60 min) Thus, neither our results nor those of other workers indicate that infused fructose is converted to any appreciable degree to glucose in the liver It appears rather as if the fructose administered replaces a large part of the normal glucose utilization in the periphery, concurrently with a decrease in the glucose output from the liver The rise in blood glucose observed in our experiments during fructose infusion indicates that the peripheral glucose consumption diminished more rapidly than the liver's glucose production Seltzer et al (31) concluded, from the results of insulin loading in combination with fructose infusion, that the fructose supplied can replace a large part of the glucose combustion in the periphery

The lactate output from the splanchnic region during fructose infusion shows that some of the fructose administered is converted in the liver to lactic acid However, in all four experiments the lactate production was much less than the fructose uptake, suggesting that a large part of the fructose supplied to the splanchnic region is stored in the liver as glycogen, or converted in some other way Similar results were obtained by Mendeloff and Weichselbaum (25)

In the experiments in which the renal net balances of fructose and glucose were also determined (by renal vein catheterization and analysis of the arterial and renal venous blood and of the urine), an output of glucose in the renal vein blood was recorded during a short part of the infusion, indicating that some of the fructose transported to the kidney brings about a production of glucose Similar results were obtained in rats by Salomon et al (30) Quantitatively, however, the renal production of glucose was of little importance

To sum up the results of the catheteri

zation studies definitely argue against infused fructose being converted to circulating glucose to any major extent Consequently, the synthesis of glycogen in muscle tissue on infusion of fructose seems to occur directly from fructose taken up in the musculature It is remarkable that the muscle glycogen formation from fructose is about the same as from glucose This is not in accordance with the mentioned experiments on rat diaphragm, which showed a much smaller uptake of fructose than of glucose, the difference being still more pronounced when the two sugars were present together in the incubation medium

The experiments in which carbohydrates were infused after work with one leg were made against the background of previous experiments (5, 21) We had then been able to demonstrate considerable stimulation of glycogen synthesis in a muscle group whose glycogen store had been exhausted beforehand by repeated hard work In these experiments carbohydrate was given orally and the glycogen was not determined until one day after exercise Consequently they provided no information about how rapidly the stimulating factor became active after depletion of the glycogen store

The present experiments with infusion of glucose and of fructose after work with one leg show that the stimulating factor comes into effect directly after the end of work (within the first 2 hours) On infusion of glucose the effect appeared to decline somewhat during the next 2 hours

In our series with glucose infusion after work with one leg the blood glucose values were consistently lower than when the subjects had rested before the infusion The lower values in the former series can be explained by the much more rapid glucose uptake in the muscles previously depleted of glycogen The significantly smaller glucose excretion in the urine during glucose infusion after work with one leg can be ascribed to the lower blood sugar concentration in this series

In the experimental series with one leg work, no significant difference was present between the urinary excretion of glucose and that of fructose during the infusion, and the net uptake of the two sugars (quantity infused minus quantity excreted) was the same (3 8 g/kg body weight)

The glucose concentration in blood during glucose infusion was, however, consistently far higher than the corresponding fructose concentration during fructose infusion It was also higher throughout than the total sugar concentration (glucose and fructose) in blood

The reason why the fructose concentration during fructose infusion rises less than the glucose concentration during glucose infusion has been considered to be that fructose is metabolized more rapidly than the corresponding amount of glucose (37, 26) Studies of the fructose concentration in the muscle of the dog and of man indicate that fructose is distributed in the intracellular fluid in unchanged form (38) This implies that it has a larger distribution volume than glucose, which is distributed in a volume comprising only about 25 % of the body weight (9) This could help to explain why the rise

in fructose in the blood is consistently much lower than the corresponding rise in glucose

In contrast to the conditions in resting muscle, the glycogen resynthesis in a muscle that has been depleted of glycogen by work seems to take place more effectively from glucose than from fructose In this respect, there is thus a distinct difference in peripheral metabolism between the two sugars

A possible explanation is that the mechanism which stimulates glycogen resynthesis locally in the muscle after work is sensitive to insulin, or that insulin is bound to the muscle that has worked Glucose infusion induces an increase of the insulin activity and insulin concentration in plasma (*1*, *15*), while intravenous fructose does not give any specific insulin stimulation (*11* *29*) Insulin has been shown to induce transformation of the enzyme glycogen synthetase from the D form (which is active only in the presence of glucose 6 phosphate) to the I-form, which is fully active even in the absence of glucose 6 phosphate (*35*) In addition, Danforth (*13*) has shown that the activity of synthetase I in rat muscle increases after emptying the glycogen store, and that the activity is inversely proportional to the glycogen content He also demonstrated that the glycogen synthesis in muscle seems to be regulated by the relation between the I and D activity Experiments are now being made with the object of ascertaining the role of glycogen synthetase in the resynthesis of glycogen after work

The lower rate of resynthesis of glycogen during fructose infusion, as compared with that during glucose infusion, might also depend on the lower total blood sugar concentration during fructose administration If the muscle blood flow is not affected differently by glucose and by fructose, the total quantity of carbohydrate supplied to the muscle (muscle blood flow x sugar concentration) would be lower on infusion of fructose, and could be a limiting factor in the synthesis of glycogen

Summary

The effect of infusion of glucose and of fructose on the synthesis of muscle glycogen was studied in healthy experimental subjects A 20 % solution of one of these sugars, representing 4 g/kg body weight, was infused in the course of 4 hours The glycogen content was determined in needle biopsy material from the quadriceps femoris muscle before, during (in two series) and after the infusion The concentration of the relevant sugar in the blood was also followed during the experiments

In two series, the subjects had rested before infusion of glucose or fructose In two other series, infusion was preceded by hard work with one leg, so that the local store of glycogen in the muscles of this leg was exhausted This was done with the object of stimulating the local resynthesis of glycogen in these muscles

In the experiments in which the subjects had not worked before infusion, both glucose and fructose produced a moderate rise in muscle glycogen, of about the same magnitude in both cases

After work with one leg the resynthesis of glycogen was considerably greater

in the leg that had worked, in connexion with infusion of both glucose and fructose This is taken to indicate that the local factor stimulating the resynthesis of glycogen comes into effect within the first few hours of ending work In the leg that had worked, the formation of glycogen was significantly higher after infusion of glucose than after that of fructose

In 4 subjects given an infusion of fructose, the liver metabolism was studied by means of hepatic vein catheterization and determination of the splanchnic blood flow with indocyanine green, as well as of the uptake and output of glucose, fructose and lactic acid Large quantities of fructose were taken up by the liver, and lactate was produced The glucose output fell gradually during infusion despite a rising arterial concentration of glucose The results indicate that in connexion with infusion of fructose the formation of glycogen takes place by a direct peripheral uptake of this sugar, and not by fructose being first converted in the liver to glucose, which is then taken up in the muscle cells

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Muscle Glycogen Synthesis in Relation to Diet Studied in Normal Subjects

By

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Bloom and Azar (7) and Azar and Bloom (2) pointed out the similarity between a carbohydrate free diet and fasting with respect to the excretion of electrolytes and to the metabolism of ketones, fat and protein They also stated that after 3 days fat diet, the experimental subjects suffered from pronounced fatigue on physical activity

In addition it has been shown that the lipid concentration in the plasma increases considerably in connexion with fasting, but that it falls on oral or intravenous administration of glucose (16) If, however, protein is given instead of glucose, it does not affect the raised lipid concentration (15)

It has long been known that the liver glycogen falls greatly during periods of starvation (8) On the other hand as far as we are aware, no previous studies have been made of the variations in muscle glycogen in man during fasting and in connexion with various diets A method has been devised at our laboratory for determining the glycogen content of needle biopsy specimens from the quadriceps femoris muscle Since repeated specimens can easily be taken, we were able to follow the variations in the glycogen content of the skeletal musculature in connexion with various diets and during starvation, as well as the resynthesis of muscle glycogen after depletion by exercise

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Material and methods

The experimental subjects consisted of 19 healthy volunteers (3 women and 16 men) aged 21—39 years With the exception of subjects G Å and P Å all those in the dietary series belonged to the hospital staff During the experimental periods none of them performed any heavy muscular work apart from that demanded by the experiment On the other hand they all carried out their customary work

Two different types of diet were used in the series as well as starvation Here, starvation denotes total abstention from calories thus the subjects were only allowed to drink water ad libitum The fat + protein diet was not standardized with respect to its fat and protein content but only as regards

its carbohydrate content This was kept below 5 % of the total caloric intake which in all subjects ranged from 30—40 Kcal/kg body weight However when comparing the fat + protein and the carbohydrate diets the caloric intake in the individual subject was the same in both types of diet

Fat + protein diet This consisted of bacon eggs meat, vegetable oils butter and small amounts of tomatoes and lettuce

Carbohydrate diet At least 95 % of the calories consisted of carbohydrates in the form of bread spaghetti, potatoes sugar, fruit and juices Spices and small amounts of fat and meat extract were added for taste

In the dietary experiments the food was cooked and controlled in the laboratory except in the case of subjects G Å and P Å

The muscle biopsy specimens were consistently taken in the morning after 12 hours fast and in certain cases in the afternoon as well

Three types of experiments were made, to study the effects listed in the following

I The effect on the muscle glycogen of a high carbohydrate diet a low carbohydrate diet, and starvation without previous depletion of the muscle glycogen by exercise

Experiments of this type were made in two healthy subjects (E H and J B) Before the experiment both ate a normal diet and performed ordinary laboratory work During the experimental period this work was continued as before but the diet was changed E H first ate the fat + protein diet for 7 days followed by the carbohydrate diet for 5 days The caloric intake was the same during both periods Biopsy specimens were taken from the quadriceps femoris muscle every other day Subject J B fasted for 5 days and the muscle glycogen content was determined in biopsy specimens at regular intervals

II The effect of different types of diet and of starvation on the resynthesis of glycogen after muscular work

A A series of 10 subjects performed continuous work on a bicycle ergometer The load was approximately 60 % of $W_{1 0}$ The muscle glycogen content was determined before work immediately after it and after 1 hour s fasting and rest

B This series consisted of 5 subjects After a muscle biopsy specimen had been taken, they performed hard intermittent work on a bicycle ergometer The work was continued to maximal muscular exhaustion i e until the subjects could not work continuously for more than 2 min with a sub maximal load A further biopsy specimen was taken directly afterwards and another 4 hours later The subjects then rested until the next morning when another biopsy specimen was taken They fasted during this first 24 hours After biopsy on the morning of day 2 the dietary experiment was started (fat + protein or carbohydrate diet)

Two subjects were given the carbohydrate diet for one day after which muscle biopsy was again performed

One subject was given the fat + protein diet for 1 day and the carbohydrate diet on the next day The caloric intake was the same on both days

Two subjects ate the fat + protein diet for 5 and 6 days respectively, followed by the carbohydrate diet for 1 day Biopsy specimens were taken daily, or every 2nd to 3rd day

III Comparison between the effect of different diets on glycogen synthesis in muscles depleted of glycog n and in muscles with intact glycogen stores in the same subject

This series consisted of four subjects The principle was that they performed work with one leg, this resulting in local emptying of the muscle glycogen store Biopsy specimens were then taken from both legs concurrently, after the end of work as well as after different types of diet for varying periods

In two cases, the first part of the period following the work comprised 2 days starvation A biopsy specimen was taken on the morning of the third starvation day after which work was performed on the bicycle ergometer

with the same leg as before The work was followed by biopsy and the carbohydrate diet was then started After 4 days on this diet biopsy specimens were again taken from both legs

The same type of experiment was done in the other two subjects except that in place of 2 days starvation the fat + protein diet was given for 3 days

The muscle glycogen was analysed in specimens from the quadriceps femoris muscle obtained by the needle biopsy technique (3) The specimen was dissected free from visible connective tissue and fat weighed on a micro-balance and homogenized in a Potter Elvehjem glass homogenizer The protein was precipitated with trichloroacetic acid and the glycogen precipitated from the supernatant with ethanol After hydrolysis in hot sulphuric acid glucose was determined by the *o* toluidine method (13) Reprecipitated and dried glycogen (National Biochemical Co Cleveland Ohio) was used as reference substance

A detailed description of the method together with normal values and variations is in the press (14) The normal value for muscle glycogen ranges from 0 95 to 2 0 g/100 g wet muscle (quadriceps femoris) the mean is 1 39 g and the error of the method in duplicate determinations is 0 05 g/100 g tissue

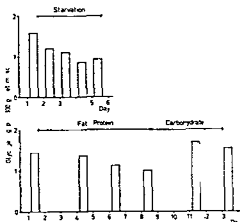


Fig 1 The effect of starvation and different diets on the muscle glycogen concentration in two normal subjects No heavy exercise was performed immediately before or during the experimental period

Results

I The results are shown in fig 1 The glycogen content of the quadriceps femoris muscle fell both on starvation (J B) and with a fat + protein diet (E H) Thus during 5 days starvation the glycogen content fell from 1 58 to 0 95 g/100 g wet muscle The fat + protein diet continued for 7 days, led to a decrease in glycogen content from 1 44 to 0 99 g/100 g wet muscle After 5 days carbohydrate diet, the concentration had risen from 0 99 to 1 54 g/100 g wet muscle

II A The mean value for muscle glycogen before starting work was 1 46 $\pm$ 0 12 g/100 g wet muscle (n = 10) directly after ending work 0 39 $\pm$ 0 061 g and after 1 hour s fasting and rest 0 40 $\pm$ 0 08 g Thus when the subjects had fasted there was no significant rise in muscle glycogen during the first hour after work

II B The results are presented in table I The mean value for muscle glycogen was 1 47 g (n = 5) before starting the experiment 0 03 g directly after work, and 0 24 and 0 37 g/100 g wet muscle after 4 and 20—22 hours starvation respectively The fat + protein diet produced an extremely slow resynthesis of muscle glycogen Thus, the initial value had not been regained even after 5—6 days (subjects EH and OA) The mean increase per day during the fat + protein diet was 0 12 g glycogen/100 g wet muscle (n = 12) The carbo

TABLE I Muscle glycogen content before and after muscular work Resynthesis after different

Subject Height (cm)	Age (yrs) BW	Sex	Glycogen content of quadriceps femoris muscle g/100 g wet muscle tissue							
			Day	1			2	3	4	5
				9 am	After work	5 pm	9 am 5 pm	9 am 5 pm	9 am	9 am
N A 178	24 68	♂	Diet	Starvation 1 60	 0 05	 0 26	F + P 0 54 0 54	CH 0 54 1 05	 1 42	
A F 163	26 49	♀	Diet	Starvation 1 43	 0 02	 0 18	CH 0 20 1 10	 2 25		
A K 175	20 66	♀		1 41	0 01	0 20	0 44 1 70	1 75		
E H 189	39 85	♂	Diet	Starvation 1 66	 0 01	 0 36	F + P 0 43 0 61	F + P 0 63	F + P 0 76	F + P 0 70
O A 182	22 75	♂	Diet	Starvation 1 24	 0 01	 0 20	F + P 0 22	F + P 0 24	F + P 0 47	F + P 0 91

[1] F + P = 2 000 Kcal of fat and protein CH = 2 000 Kcal of carbohydrate

ydrate diet, on the contrary, resultled in a rapid increase in the muscle glycogen store to values which, in three cases, greatly exceeded the initial value The mean increase per day was 1 20 g/100 g wet muscle (n = 5)

hII The results in this series are given in figs 2 and 3 During two days' starvation, the muscle glycogen concentration in the leg that had not worked fell from 1 23 to 0 67 g/100 g muscle (S K) and from 1 29 to 0 92 g (A S K) In the leg whose glycogen store had been depleted by work, the value rose during the same period from 0 04 to 0 28 g (S K) and from 0 09 to 0 46 g A further period of work with the leg that had worked before decreased the glycogen content to 0 06 and 0 22 g/100 g wet muscle, respectively

Four days carbohydrate diet increased the glycogen content of the leg that had not worked from 0 67 to 1 63 g/100 g wet muscle (S K) and from 0 92 to 1 83 g (A S K) In the leg whose glycogen store had been emptied by exercise, the rise was of a completely different order of magnitude, i e , from 0 06 to 3 30 g/100 g wet muscle (S K) and from 0 22 to 3 47 g (A S K)

In the other two subjects (G Å and P Å) — who instead of fasting for 2 days ate a fat + protein diet for 3 days — the changes in muscle glycogen were of

diets

6	7	8	9
	9 am 5 pm	9 am	9 am
F + P	CH 1 02 1 61		
F + P	F + P	CH 1 10	2 11

essentially the same type Thus, the fat + protein diet was associated with a decrease in the glycogen content of the leg that had not worked from 1 16 to 0 85 g/100 g wet muscle (G Å) and from 1 24 to 0 80 g (P Å) In the leg that had worked, the content rose from 0 09 to 0 39 g/100 g wet muscle, and from 0 06 to 0 45 g respectively

The exercise performed on the morning of the fourth day reduced the muscle glycogen content from 0 39 to 0 16 g/100 g wet muscle (G Å) and from 0 45 to 0 03 g (P Å) During the subsequent period on a carbohydrate diet, the glycogen concentration in the leg that had not exercised rose from 0 85 to 1 70 g/100 g wet muscle and from 0 80 to 1 07 g respectively In the leg that had exercised, the corresponding rise was from 0 16 to 3 22 g/100 g wet muscle and from 0 03 to 2 49 g respectively

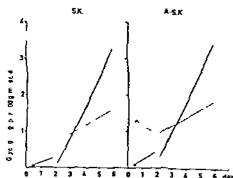


Fig 2 Effect of starvation and of a carbohydrate rich diet on the glycogen concentration in the quadriceps muscles of two normal subjects Exercise with one leg was performed immediately before the first biopsies After two days of starvation further exercise with the same leg was performed and thereafter a carbohydrate rich diet was given ———— worked leg – – – – – rested leg

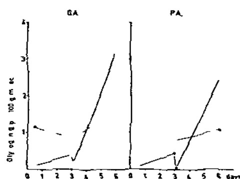


Fig 3 Effect of a carbohydrate free and of a carbohydrate rich diet on the muscle glycogen concentration in two normal subjects The same type of experiment as in figure 2 but instead of 2 days of starvation 3 days of fat and protein diet was instituted between the two one leg work periods ———— worked leg – – – – rested leg

Discussion

It is known from the studies of Christensen and Hansen (9) that an individual s ability to perform prolonged work is closely related to his diet before the exercise test The authors demonstrated that a low carbohydrate diet results in much poorer endurance than a high carbohydrate diet

We have previously shown, in our laboratory, that the muscle glycogen concentration falls during exercise (5), and that a subject's working capacity is correlated to the glycogen content of the working muscles (1) The present investigation was made with the object of ascertaining the extent to which the diet can affect the storage of glycogen in the skeletal muscles We were able to demonstrate, in a preliminary study, that the synthesis of glycogen in con nexion with a carbohydrate diet differed according to whether the glycogen store was intact or greatly depleted at the beginning of the dietary period (6) Thus, the resynthesis of glycogen was highly stimulated locally in a muscle group which, before administration of carbohydrate, was depleted of glycogen by exercise The glycogen concentration in this muscle group did in fact, rise far above that in corresponding muscles that had not worked

In order to investigate in greater detail the importance of nutrition in the glycogen metabolism of the muscles, we made three types of dietary experiments In one group of experiments the glycogen depots were intact, and in another they were depleted by muscular work In a third series, the two experimental models were combined by reducing the glycogen store in one leg (by work on a bicycle ergometer), whereas that in the other leg remained intact

It is evident from the results that a low-carbohydrate diet and starvation had the same qualitative effect on the glycogen content of the muscles When the dietary experiment was started with intact glycogen depots, the concentration fell slowly, despite the fact that no heavy muscular work was performed This implies that, in this situation, the gluconeogenesis (from fat and protein) is not rapid enough to maintain the muscle content of glycogen even when only extremely light, intermittent work is carried out

With a high carbohydrate diet, on the contrary, the glycogen level rises above the basal value

There is thus a marked difference between a high carbohydrate and a low carbohydrate diet with respect to their ability to maintain the glycogen store of the skeletal muscles However, with both types of diet, as well as with starvation, the changes in glycogen concentration were moderate, and the glycogen level tended to stabilize after a few days on the different regimes

An observation which applies both to starvation and to the various diets is that depletion of the glycogen depots by muscular work stimulates glycogen synthesis With a low carbohydrate diet, however, this resynthesis is exceedingly slow, and complete restitution of the glycogen content is delayed for several days, up to a week With the high carbo hydrate diet, on the other hand, the resynthesis is extremely rapid Moreover,

the concentration of glycogen rises far above the normal range

It is apparent from our present experiment with one leg exercise (figs 2 and 3), as well as from our preliminary study (6), that the factor stimulating the resynthesis of glycogen is a local one, and is bound within the muscle group emptied of its store Consequently, the factor cannot be identical with the circulating substance described by Goldstein (12)

The nature of this glycogen stimulating principle in the glycogen depleted muscles is unknown Danforth (10) showed that a transformation of glycogen synthetase from the I (independent) into the D (dependent) form takes place on depletion of the glycogen store The I form — which arises by dephosphorylation of the corresponding D form (11) — is active in the absence of glucose 6 phosphate (independent), and would be able to hasten the resynthesis of glycogen from uridine diphosphate glucose This would result in a rapid increase in the glycogen store This transformation from the D form into the I form is stimulated by glycogen depletion, as well as by insulin, but is retarded by adrenaline (10) In preliminary experiments in man, we observed a corresponding shift of the glycogen synthetase activity towards the I form in glycogen depleted muscle, this I form activity ranging from 30 % in muscles with intact glycogen stores up to 80 % of the total activity in depleted muscle tissue This increase does not, however, persist for long enough to be the sole explanation of the muscle glycogen resynthesis, which in these experiments has been shown to proceed for several days

It is well known that the blood flow increases in working muscles, and that this increase can persist for some time after work This would mean an increased glucose load to the 'worked' muscle group during the time when the blood flow is high This augmentation, however, will persist only for some minutes up to an hour, and thus cannot explain the difference in glycogen synthesis between the two muscle groups which we found to persist for several days

A diet rich in carbohydrates will produce an increased output of insulin from the pancreas This increase per se cannot explain our results, but a local stimulation of insulin activity, or a local binding of insulin in 'worked' muscle, could be the operating factor

Further work is now being done to elucidate the nature of the factor which stimulates the synthesis of glycogen

In another study (4), the capacity to perform long term heavy exercise was found to be correlated to the muscle glycogen content varied by means of exercise and diet regimens

Summary

The glycogen concentration in the quadriceps femoris muscle in man was studied by analysis of needle biopsy material Biopsy specimens were taken in connexion with various diets, as well as before and after periods of hard muscular work

Three forms of dietary regimen were compared, i e, starvation, a normo-

caloric diet consisting of fat + protein, and a high carbohydrate diet with the same caloric content The following results were obtained

I Starvation and a low carbohydrate diet had essentially the same effect on the glycogen content of the muscle, i e, a fall in the level from the initial value After depletion of the glycogen store locally by muscular work, a resynthesis of glycogen took place It was, however, extremely slow, and the initial value had not been regained after one week's full caloric diet

II A high carbohydrate diet raised the muscle glycogen level above the normal value, and produced exceedingly rapid resynthesis after depletion of the glyco gen store A marked rebound effect was observed Thus, after depletion, the glycogen concentration was found in most cases to exceed the normal range for the quadriceps femoris by more than 100 %, when the high carbohydrate diet was continued for several days

III The factor stimulating glycogen resynthesis, which became operative after emptying of the store by muscular work, is shown to be local, and to be bound within the muscle group depleted of glycogen

Acknowledgements

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Adrenergic Beta-blockade and Physical Working Capacity

By

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It has recently been shown that there is a varying sympathetic tone in patients with various psychiatric diseases and that this autonomic imbalance influences the relationships between physical working capacity and the circulatory dimensions heart volume and the total amount of hemoglobin (9) During an adrenergic beta receptor blockade there was a more or less pronounced increase in the physical working capacity at pulse 170 and the above-mentioned relationships did not differ from those in healthy subjects

Arvedson et al (2) suggested that an increased sympatho-adrenal activity was one patho-physiological mechanism in patients with vasoregulatory asthenia (VA) (12) These patients are characterized by a low physical working capacity in relation to circulatory dimensions (blood and heart volume)

The most important nervous transmittor of the adrenergic beta receptors in humans is adrenaline (1) An increased urinary excretion of this substance is common among patients with psychiatric diseases (3, 5, 17) It may also occur during emotional stress among healthy subjects, e g air force personel (6) and boxers (5)

It appears that autonomic disturbances with increased activity or tone of the sympathetic system may occur in patients with different working capacity and physical activity It is probable that a high sympathetic tone is encountered in cases of VA and a vagotonia or low sympathetic tone among athletes This study was carried out to test this theory by examining during a work test the effect of an adrenergic beta receptor blocking agent on subjects with a low ordinary or high physical working capacity at pulse 170 in relation to heart volume and the total amount of hemoglobin

Material

The material consisted of subjects examined at the Department of Clinical Physiology over a period of 1 1/2 years They were either patients sent from different departments of the hospital or volunteers From

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TABLE I Mean (M) and standard deviation (S D) of some anthropometric data pulse rates at of VA patients (VA) control subjects (C) and athletes (A)

		No		Age (yrs)	Height (cm)	Weight (kg)
VA	♀	3	M	25	166	63
	♂	8	M	31	179	74
			S D	±14	±6	±4
C	♀	10	M	36	166	59
			S D	±11	±6	±9
	♂	8	M	30	176	64
			S D	±14	±7	±6
A	♂	12	M	19	180	69
			S D	±1	±5	±6

these cases three groups of subjects were selected, those with low ordinary or high physical working capacity at pulse 170 (W_{170}) in relation to heart volume (HV) and the total amount of Hb (THb) The $W_{1\,0}$ was considered to be normal when its relation to HV and THb was within ± 1 S D from the regression lines between $W_{1\,0}$ — HV and $W_{1\,0}$ — THb described by Holmgren et al (13) A W_{170} more than + 1 S D from the regression line was considered as high and a W_{170} below — 1 S D as low

Group VA

This group included all subjects with a low $W_{1\,0}$ *in relation to HV and THb* Cases with clinical signs of thyrotoxicosis or conditions other than VA known to be associated with a hyperkinetic circulation or other somatic diseases were excluded Eleven patients fulfilled these criteria three women and eight men They had a high pulse rate at rest and during an orthostatic test (table I) Some of them also had sympathetic ST T changes in the ECG at rest during the orthostatic test and during work These ECG changes disappeared during an adrenergic beta receptor blockade (8) These patients may be regarded as cases of vasoregulatory asthenia (group VA) (12)

Group C

Criteria for inclusion in this control group were an ordinary $W_{1\,0}$ in relation to HV and THb ordinary daily physical activity *and absence of signs of somatic disease* Eighteen subjects (ten women and eight men) fulfilled them The subjects had an *ordinary or somewhat high pulse rate at* rest and during the orthostatic test (table I) They had no ECG changes except for some cases with slight functional ST T changes especially during the orthostatic test These subjects formed the control group (group C)

Group A

This group was selected from conscripts sent to the laboratory to estimate their physical working capacity Twelve of them were healthy and had a high $W_{1\,0}$ in relation to HV and THb They had a low pulse rate at rest and during the orthostatic test (table I) Ten of them had normal ECG recordings One had some supraventricular extrasystoles and in another an AV block of the first degree was recorded after the work test

Six of the cases in this group took part in the Swedish military championships in ski shooting a competition comprising both

rest and in the standing position and the dosage of propranolol per kg body weight in the groups

Heart vol (ml)	Total amount of Hb (g)	Pulse rate at rest	Beats/min standing	Dosage of propranolol/kg body weight (mg)
613	533	99	126	0 24
824	782	91	117	0 23
±93	±86	±30	±23	±0 03
572	519	86	104	0 23
±99	±68	±12	±14	±0 02
716	663	79	102	0 20
±73	±93	±11	±11	±0 03
838	761	60	73	0 22
±121	±80	±7	±14	±0 02

cross country skiing and shooting All subjects had normal chest X rays A heart volume slightly larger than 500 ml/m² body surface area was found in two athletes Enlargement of the heart has been considered to be a physiological adjustment to work in subjects exposed to physical training (15)

They all had normal blood pressure The total amount of Hb was in no case less than 20 % or the value predicted from the body weight The heart volume and the total amount of hemoglobin were not determined during the blockade They were supposed to be of the same magnitude as before Some anthropometric data are given in table I

Methods

The methods for determining the physical working capacity heart volume and the total amount of hemoglobin were identical with those used in a previous study (9) The subjects performed a standardized work test before and one hour after oral administration of a beta adrenergic blocking agent propranolol (Inderal®) The second test was generally performed within a week of the first During an orthostatic test that preceded the work tests the pulse rate and the ECG were recorded after 8 min in the standing position The dosage of the drug was determined by body weight Ten mg was given to subjects weighing less than 60 kg 15 mg to those between 60 and 75 kg and 20 mg to those exceeding 75 kg The dosage/kg body weight was similar in all groups (table I) The physical working capacity was calculated at pulse rate 170 beats/min by inter or extrapolation assuming a linear relationship between pulse rate and work load (W_{170})

The work load at maximal working intensity (W_{max}) was taken to be the heaviest load at which the subject worked for 6 min with an increment proportional to the completed part of the period at the next highest load

Conventional statistical methods were used for calculating the arithmetic mean and the standard deviation of the mean The significance of differences between mean values was tested by Student s t test (16) The Spearman rank correlation coefficient was used for testing the significant limits of correlation (14)

TABLE II Means of physical working capacity at pulse 170 (W_{170}) and maximal working capacity (W_{max}) before (O) and during (P) adrenergic beta blockade and differences between the means in VA patients control subjects (C) and athletes (A)

	No		W_{170}			W_{max}		
			O	P	diff P—O	P	P	diff P—O
VA ♀	3	M	411	719	308	533	567	34
C ♀	10	M	638	819	181	600	650	50
		S D	±186	±281	±119	±138	±83	±112
VA ♂	8	M	684	1237	553	844	1000	156
		S D	±235	±224	±261	±172	±185	±232
C ♂	8	M	906	1109	203	988	1050	63
		S D	±105	±182	±114	±113	±101	±104
A ♂	12	M	1547	1691	144	1733	1704	—29
		S D	±133	±269	±197	±114	±157	±127

Results

Effect of propranolol on W_{170} and its relationships to HV and THb respectively

Group VA After the blockade the mean W_{170} was almost doubled in the VA group and the relative increase was almost the same for both sexes The mean values increased from 411 to 719 kpm/min in the females and the corresponding mean values for the males were 684 and 1237 kpm/min (table II) During the blockade there was generally a return to normal of the relationships between W_{170} and the circulatory dimensions HV and THb The most marked effect of propranolol was generally found in the VA cases with the lowest W_{170} in relation to HV and THb before the blockade (fig 1)

Group C The relative mean increase in W_{170} during the blockade was about 1/3—1/4 of that in group VA The mean W_{170} increased after propranolol from 638 to 819 kpm/min in the women and from 906 to 1109 kpm/min in the men (table II) The mean increase in W_{170} during the blockade was significantly lower in the male control subjects than in the male VA-patients (table III) The individual changes of W_{170} during the blockade varied and this is shown in fig 1 The standard deviation of the mean increase in W_{170} was almost identical for both sexes

Group A The mean increase in W_{170} during the blockade was less in the athletes as compared with the control subjects and VA patients The difference between group A and VA was statistically significant, while insignificant between group A and C (table III) There was a higher standard deviation for the mean increase in W_{170} in group A than in group C This was mostly due to a few cases among the athletes, which is apparent from fig 1

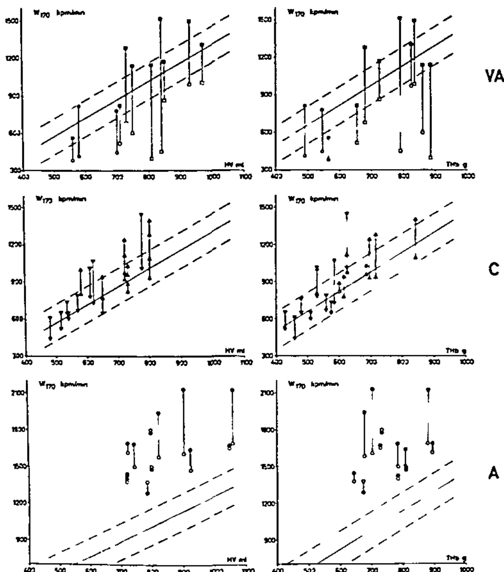


Fig 1 The relationships between physical working capacity at pulse 170 (W_{170}) and heart volume (HV) and the total amount of hemoglobin (THb) respectively before (unfilled symbols) and during (filled symbols) adrenergic beta receptor blockade in patients with vasoregulatory asthenia (VA) control subjects (C) and athletes (A) Regression line ±1 S D from 58 healthy subjects of various age sex and degree of physical training (13)

Effect of propranolol on W_{max}

A mean increase in W_{max} of 156 kpm/min was recorded among the male VA patients during the blockade This change is probably significant ($p < 0.05$) The corresponding increase in the male control subjects was 63 kpm/min (table II) W_{max} was 29 kpm/min

TABLE III Differences between the male groups of VA patients control subjects (C) and athletes (A) in mean increase in physical working capacity at pulse 170 during an adrenergic beta receptor blockade

	VA—C	VA—A[1]	C—A[1]	C—A[2]
Difference (kpm/min)	+350	+409	+59	+181
P	<0.01	<0.001	>0.05	<0.01

[1] n=12 [2] n=7 (see text)

lower after propranolol than before among the athletes This effect of the drug on W_{max} in the athletes is different ($p < 0.05$) from that in the male VA patients, where an increase was recorded No statistically significant difference in change of W_{max} was noted when comparing VA patients and control subjects or control subjects and athletes

Orthostatic test and the effect of propranolol on W_{170}

There is a significant correlation between the increase in W_{170} during the blockade and the level of the pulse rate during the orthostatic test ($p < 0.001$) and between the increase in W_{170} and the increase in pulse rate from rest to after 8 min standing ($p < 0.01$) These calculations were made in the male groups

An increase in pulse rate of more than 16 beats/min during the orthostatic test was common among the VA patients and also among the control subjects A similar orthostatic pulse reaction was also recorded in five athletes who had a mean increase in pulse rate during the orthostatic test of 25 beats/min compared to four in seven other athletes These five cases also had a mean increase in W_{170} during the blockade of 316 kpm/min, i e of a magnitude between that found in the VA patients and that of the controls The corresponding mean increase in W_{170} after propranolol among the rest of the athletes was 22 beats/min If the five athletes with a high increase in pulse rate during the orthostatic test were withdrawn from group A a statistically significant difference emerged between this remaining group of athletes (A^2) and the control subjects in the mean increase in W_{170} during the blockade (table III)

Sympathetic tone and performance of athletes

Six of the athletes took part in the Swedish military championships in ski shooting In three of them there was a high increase in pulse rate during the orthostatic test and a pronounced effect of propranolol on W_{170} while the corresponding changes were smaller in the others The results obtained from these small groups during the cross country skiing did not differ, while there was a surprising difference in the shooting performances The former cases had 6 4 and 3 missed shots, while the latter had 2 0 and 0 respectively Altogether

15 shots were fired at three ranges during a cross country ski run covering 15 km

Side effects

There was a noticeable effect of propranolol on two athletes Before the drug was given they had a heart rate of 57 beats/min at rest During the blockade, periods of partial sinoauricular block with nodal escape beats were recorded at rest This arrhythmia disappeared during work

Discussion

The results indicate that a study of the relationships between physical working capacity at a given pulse rate and the circulatory dimensions, heart volume and the total amount of Hb, is valuable for estimating the degree of sympathetic tone If the effect of the adrenergic beta receptor blockade on W_{170} is taken as a sign of sympathetic tone during the work test this tone was most pronounced in the group of VA patients They were characterized by a low W_{170} in relation to HV and THb The lowest tone was encountered in the group of athletes who had a high W_{170} in relation to circulatory dimensions Healthy control subjects with an ordinary W_{170} in relation to HV and THb had a sympathetic tone during the work test that was somewhere between the VA patients and the athletes These statistical calculations are valid for male VA-patients, control subjects and athletes viewed as groups There is no reason to believe that the female groups would react differently

Judging from the results some circulatory changes similar to those in VA seem to exist among *certain* control subjects and athletes In these latter groups subjects were found with an increase in W_{170} during the blockade of almost the same magnitude as in VA patients This reaction was associated with a relatively high pulse level and a relatively marked increase in pulse rate during the orthostatic test i e findings typical of VA

Treumann and Schroeder (18) reported a high muscle blood flow in the forearm at rest in some boxers before a fight The values were of the same magnitude as those found in VA patients at rest (11)

An interesting observation found in three athletes is the correlation between pulse level and pulse reaction during the orthostatic test the effect of propranolol on W_{170} and the results from the ski shooting Elmadjian et al (5) have reported that *certain* boxers have higher urinary excretion before a fight than afterwards Treumann and Schroeder in their study reported that a high muscle blood flow at rest had an unfavourable influence on athletic performance It is possible that there is an individual disposition to nervous tension among athletes and that such a factor may explain the observed correlation It is also possible that the methods used in this study may be of value in athletic and military research for revealing subjects with autonomic instability

Furberg in 1965 (7) suggested an increased beta adrenergic tone to be one patho physiological mechanism in the VA syndrome The same year Bollinger et al

(4) reported that the physical working capacity *increased after* propranolol in cases with a low working capacity in relation to THb, while a comparatively smaller increase was found in healthy subjects and patients with thyrotoxicosis These results are in accordance with those presented in this study

Predicting the sympathetic tone with the aim of calculating the relationships between circulatory dimensions may be of clinical value Furberg and Jacobsson (10) have found that the beneficial effect of propranolol in treatment of patients with angina pectoris is positively correlated to the difference between determined physical working capacity and that estimated from the heart volume

Summary

The effect of an adrenergic beta receptor blockade induced by propranolol was studied in three groups of subjects during a work test The investigated groups consisted of subjects with a low, ordinary or high physical working capacity at pulse 170 (W_{170}) in relation to the circulatory dimensions, heart volume and the total amount of Hb

Patients with a low physical working capacity, interpreted as cases of vasoregulatory asthenia, almost doubled their W_{170} after propranolol (about 550 kpm/min for males) The maximal working capacity was about 150 kpm/min higher during the blockade In control subjects with ordinary W_{170} the effect of propranolol was less The mean increase in W_{170} during the blockade was about 20 % (200 kpm/min for males) and the corresponding increase in W_{max} was about 60 kpm/min In athletes the mean increase in W_{170} was less than 10 % (about 100 kpm/min for males) and the maximal working capacity decreased slightly in most cases

A positive correlation was recorded between the increase in W_{170} during the blockade and the orthostatic pulse rate (before the blockade) and between this increase in W_{170} and the increase in pulse rate during the orthostatic test

In five of the athletes there was a marked increase in pulse rate during the orthostatic test and a high increase in W_{170} during the blockade It seems as if some changes of vasoregulation typical of VA patients may be found in subjects with a high physical working capacity It is interesting to note that three of these five cases had four times as many missed shots during a ski shooting competition as three of their comrades whose pulse rate during the orthostatic test and whose W_{170} during the blockade only increased slightly

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(4) reported that the physical working capacity increased after propranolol in cases with a low working capacity in relation to THb, while a comparatively smaller increase was found in healthy subjects and patients with thyrotoxicosis These results are in accordance with those presented in this study

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From the Clinic for Infectious Diseases Karlstad and the National Bacteriological Laboratory, Stockholm, Sweden

Serum Hepatitis among Swedish Track-finders

II A clinical study

By

Gunnar Gille, Olof Ringertz[1] and Bo Zetterberg

During 1957—1962 an outbreak of hepatitis involving 568 cases occurred among Swedish track finders In an epidemiological study of this epidemic (6) the length of the incubation period of the illness was found to be 90—150 days The investigations also revealed the presence of a mechanism of inoculation These findings strongly suggested that the disease was serum hepatitis caused by virus B

In the absence of specific virological methods the differential diagnosis between infectious hepatitis (IH) and serum hepatitis (SH) is doubtful in individual cases The two forms of hepatitis, however display certain characteristic clinical features which may give additional support to the diagnosis, especially in epidemiologically homogeneous material

Thus a SH infection has a more insidious onset than an IH infection In the former infection, general systemic symptoms appear less frequently and the rise of temperature generally does not exceed 38° C (5, 10) Later in the course of the disease the two forms of hepatitis cannot be distinguished clinically, nor do pathological investigations such as liver biopsies, contribute to the establishment of a correct differential diagnosis Therefore, a thorough epidemiological investigation is essential in every case of hepatitis One should keep in mind the statement by Sherlock that any patient developing jaundice within six months of any procedure involving puncture of the skin must be assumed to have contracted serum hepatitis until proved otherwise (8)

The mortality rate for IH infection is considered to be one or two per 1 000 cases (11), whereas according to most statistics the rate of SH infection is considerably higher, from 0 5 % up to 20 % (7) In an epidemiological investigation of serum hepatitis following blood trans

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fusions, Allen and Sayman (1) found 13 deaths in 77 cases No deaths occurred, however, among the 21 patients who were under 40 years of age Undoubtedly the age and state of health of a person who contracts a SH infection are of great prognostic significance This relationship has been pointed out by several authors

The purpose of this study was to establish whether the clinical symptoms of track finders hepatitis coincided with the usual picture of serum hepatitis, thus excluding other causes of jaundice such as *toxic hepatitis*

Material

Altogether 345 cases of hepatitis are known to have occurred among track finders during the period Dec 1957 — May 1961 Three hundred and twenty six of the cases were hospitalized This investigation is based on the case histories received from 320 of these patients The age distribution of these cases is similar to the whole material of 345 cases mentioned above Very little is known about the clinical picture of the 19 cases that were not hospitalized Perhaps these cases were not brought to the hospital because they showed only mild symptoms There may also have been a number of sub-clinical cases

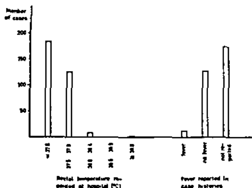


Fig 1 Rise in body temperature (320 cases)

During most of the 3 1/2 year period the track finders were aware of the symptoms of hepatitis It may be assumed therefore, that almost all cases exhibiting jaundice were recognized as hepatitis and hospitalized

The records were collected from a large number of hospitals in Sweden but there were considerable differences in the way these records were kept especially with regard to the case histories Numerical data such as body temperature and laboratory results were recorded more uniformly, although not all laboratory tests were carried out in all cases SGOT or SGPT estimations, thymol turbidity tests and serum bilirubin estimations were carried out in 210 cases and in 74 of them an electrophoretic investigation of serum was made as well

Results

Symptoms and signs

Prodromal symptoms

Eleven % of the patients suffered from arthralgia and muscular pains during the pre icteric stage of the disease and 11 % from headache

Temperature

A rise of temperature was reported by 13 of the patients, while 130 denied this symptom (fig 1) In the remaining 177 cases no information was available Since fever is a symptom that patients are questioned about and in general do not forget to mention, it is probable that there was no significant pyrexia in these cases

In hospital the rectal temperature was usually checked twice daily Only ten patients (3 %) had a temperature exceeding 37 9° C, two of them above 38 4° C

Jaundice

Jaundice was naturally the dominant symptom Eighty nine % of the patients

had noticed that their urine was dark coloured, 98 % observed jaundice and 51 % discolouration of faeces With the onset of jaundice anorexia occurred in 71 % and marked fatigue was felt by 73 % of the patients, while pain in the epigastrium was reported by 34 % and itching by 14 %

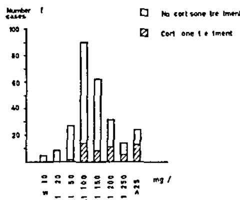


Fig 2 Maximum bilirubin level (261 cases)

Laboratory findings

Serum bilirubin

Bilirubinaemia is of special interest as an index of the intensity and duration of the disease Tests for bilirubin in serum were done in 261 of the cases (4) Fig 2 shows that in 69 % of the cases the bilirubinaemia was moderate — between 2 1 and 15 0 mg %, while in 27 % it ranged from 15 1 to over 25 mg % In only 4 % of the cases were values less than 2 1 mg % found Cortisone treatment was given in 53 of the cases, most of them showing high bilirubin values

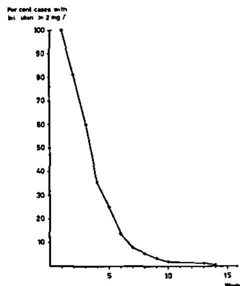


Fig 3 Duration of bilirubinaemia (163 cases)

In 203 cases the serum bilirubin level was estimated several times during the course of the disease The duration of the bilirubinaemia in 163 cases not treated with cortisone is illustrated in fig 3 In about 8 % of the cases it lasted for seven weeks or more In 18 cases where cortisone treatment was given as a routine on admission, as well as in 22 cases where treatment was given because of the severity of the disease the return of the bilirubin level to normal was accelerated As may be surmised from fig 4 there was a relationship between the intensity and duration of jaundice, the more intense the jaundice the longer the duration of the illness

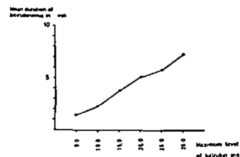


Fig 4 Relation between maximum level of bilirubin and duration of bilirubinaemia (163 cases)

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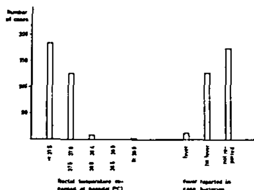


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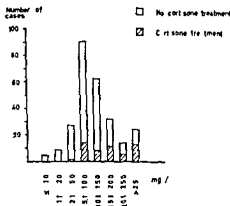


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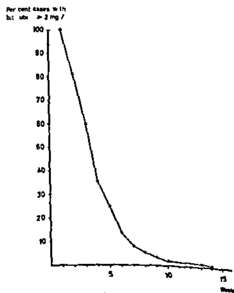


Fig 3 Duration of bilirubinaemia (163 cases)

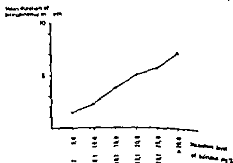


Fig 4 Relation between maximum level of bilirubin and duration of bilirubinaemia (163 cases)



Fig 5 Maximum transaminase level

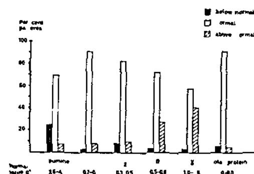


Fig 6 Serum electrophoresis (91 cases)

Fig 7 Maximum level of thymol turbidity (158 cases)

Serum transaminase

An increase of SGOT and SGPT is a characteristic feature of hepatitis values between 500 and 1 000 carmine units are usual (12, 13) but considerably higher values are frequently encountered Transaminase peaks appear early in the disease and if it is not until a later stage that the patient comes for examination a return to normal may already have begun As shown in fig 5 most of the track finders exhibited high values

Serum electrophoresis

Total serum protein levels usually remain unchanged in hepatitis However, electrophoretic investigation of the serum frequently reveals a relative decrease in serum albumin and an increase in β and γ globulin fractions As variations in technique may influence the results use was made of the criteria for normal values set up by the different laboratories where the tests were carried out The variations were however small and the limits given in fig 6 are those most frequently applied

Paper electrophoresis was carried out in 91 cases The majority of these had normal values Nevertheless 24 % displayed a decrease in serum albumin 26 % an increase in β globulin and 41 % an increase in the γ globulin fractions

Thymol turbidity

The results of the thymol reaction are given in fig 7 Since different laboratories use different factors for conversion of extinction to thymol units we have thought it more expedient to give the extinction values If an extinction value of 0 10 is taken as the highest permissible normal value 78 % of the cases showed values above this limit

OTHER LABORATORY INVESTIGATIONS

Alkaline phosphatase activities were slightly elevated The reaction was car

ried out in 302 cases and 277 (92 %) of these had values under 20 units

In general the ESR was low In 98 % of the patients it did not exceed 30 mm

Liver biopsies were carried out in seven cases Three of these were made with the fine needle technique, and consequently the sample gave no information about the architecture of the liver These biopsies however, indicated acute inflammatory parenchymal damage with abundant occurrence of neutrophil leukocytes and lymphocytes among the liver cells In four cases biopsies were performed with the Vim Silverman technique (9) A photomicrograph from one of these biopsies made at the end of the acute stage is shown in fig 8 The liver cells, especially those located centrally in the lobuli, displayed considerable swelling with a balloon like appearance and vacuolation a few cells were degenerate Among these cells there were abundant intercellular bile pigmented layers and in the same region small bile thrombi Between the lobules of the liver there was a histiocytic infiltration which in some

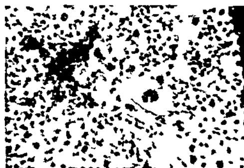


Fig 8 Photomicrograph of liver Biopsy from case 757/60 (see fig 9)

places was mixed with lymphocytes and leukocytes There was no sign of cirrhosis The histological picture was in good agreement with that of hepatitis and showed no special characteristics or atypical features

In order to illustrate the course of the disease some of the features of a typical case are given in fig 9

Mortality rate

In the 320 cases described above there was one death The patient was a 25 year old forest worker who had pre-

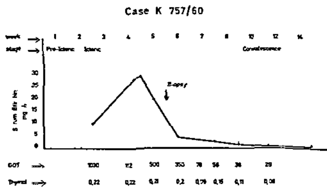


Fig 9 Course of the disease in a typical case of track finder s hepatitis

viously been healthy He died in hepatic coma after one month s illness He was severely jaundiced (bilirubin 44 mg %) and his SGOT level was very high (> 6,000 units) The histological picture of the liver indicated sub acute hepatitis

Discussion

The clinical symptoms, laboratory results and morbid anatomy of the disease described above corresponded in all respects with those of hepatitis Accordingly no other diagnosis will be discussed here

General systemic symptoms were scarce, onset was insidious and fever was mild, which fits very well with a diagnosis of serum hepatitis Since, according to part I of this study (6), the incubation period varied between 90 and 150 days it seems reasonable to conclude that the track finders' disease was serum hepatitis with an unusual inoculation mechanism On the basis of a smaller group of patients which also represents part of our material Gabinus and Jonsson (2 3) reached the same conclusion

At the time of writing 568 cases of hepatitis among track finders are known to have occurred No further death has been reported These track finders may be regarded as a group infected almost completely with serum hepatitis It is interesting to note that in this group consisting of healthy men in the ages of 20—40 years, the death rate was only one in over 500 cases, which accords with the results obtained by Allen and Sayman (1)

Summary

During 1957—1962 an outbreak of hepatitis involving 568 cases occurred among Swedish track finders Epidemiological data suggested that the disease was serum hepatitis The clinical features of the disease were studied in 320 cases

The onset of the disease was found to have been insidious in most cases A temperature of 38° C or more was found in only 3 % of the cases

The clinical symptoms, laboratory results and morbid anatomy corresponded in all respects with those of viral hepatitis Only one case was fatal

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TABLE I Age and sex

Yrs	No	Female	Male
0— 4	2		
5— 9	21		
10—14	19		
15—19	17		
20—29	5		
30—42	6		
Total	70	30	40

TABLE II

A Discovery of murmur		
Group		No
I	Discovery on routine health check up	41
II	Other casual discovery	19
III	Discovery on examination for possible heart symptoms	10
	Total	70

B Symptoms		
Group	I+II	III
Without symptoms	38	
With symptoms	22	10
Dyspnea	9	7
Fatigue	6	4
Palpitations	2	4
Chest pain	3	7
Cyanosis	2	2
Frequent respiratory infections	2	4

to use those tracings where the murmur was most dominant and most clearly outlined

During right heart catheterizations pressures were measured with an Elema variable inductance transducer and electromanometer and were recorded on a Mingograph ink jet recorder The fourth intercostal space in the anterior axillary line was used as reference level (18) We considered as upper limits for normal pressures
Right atrium mean 4 5 mm Hg
Pulmonary artery wedge mean 8 mm Hg
Right ventricle peak systolic 30 mm Hg
Pulmonary artery mean 16 mm Hg
Right ventricle — pulmonary artery systolic gradient 10 mm Hg
A pulse pressure of more than 18 mm Hg in the pulmonary artery, combined with a diastolic gradient from the pulmonary artery to the right ventricle of less than 4 mm Hg was considered to suggest incompetence of the pulmonary valve (19)

Oxygen saturation analyses were carried out in blood samples from the pulmonary and femoral arteries right ventricle, right atrium and superior caval vein *In patients* above 6—7 years of age expired air was collected in a spirometer The CO_2 and O_2 content was analyzed in a micro Scholander apparatus The cardiac output was calculated by the Fick principle

In 53 cases hydrogen curves were recorded from the platinized platinum electrode on the tip of the catheter, this being placed in the superior caval vein and in a pulmonary artery wedge position for negative and positive control respectively and in the pulmonary artery to detect any left to-right shunt

Results

Tables I and II show that about 81 % of the cases with physiological murmurs were between 5 and 19 years of age and that about 59 % of the murmurs were discovered on routine health examinations However, in many of the patients with some kind of accidental discovery of the murmur, symptoms referring to the heart and circulation were present Almost one half of all the patients had such symptoms One patient had bronchial asthma In the others exertional dyspnea and palpitations could be explained most often by lack of training,

TABLE III Localization and strength of systolic murmur

Area of maximal intensity					Total no	Strength				Total no
Left intercostal spaces						Grades 1—6				
1st	2nd	3rd	4th	Apex		1	2	3	4	
2	38	16	9	5	70	5	25	39	1	70

TABLE IV Length and shape of the systolic murmurs on the phonocardiograms Age distribution of different types

Shape	Lengths (% of S1—S2 interval)			No	Year groups			
	40—59	60—69	80—100		0—9	10—14	15—19	20—42
1 Crescendo-decrescendo	3	20	13	36	11	11	9	5
2 Decrescendo	5	8	3	16	6	3	4	3
3 Mixture or changing between 1 and 2	2	1	7	10	4	3	2	1
4 Spool shape holosystolic				5	1	2	1	1
5 Varying length and shape				2			1	1
6 Continuous systolic diastolic				1	1			
Total				70				

as these patients were leading an inactive life Influenced by the discovery of the heart murmur, the patients reckoned the chest pains to be mainly stabbing or stitch like The cyanosis was present only in cold weather

In most of the cases the murmur was best heard in the 2nd or 3rd left inter spaces (table III) In 12 cases the mur mur was noted to be weaker in the sitting position. Negative information on this point, however was usually lacking and the number may be a minimum The intesity of the murmur in about 58 % of the patients was stronger than grade 2 The 2nd pulmonic sound was described as accentuated in 13 cases In 15 cases the 2nd sound was considered to be constantly split One patient had a systolic diastolic continuous murmur over the upper part of the precordium which proved to be a venous hum In two patients a venous hum was found in the neck in addition to the precordial systolic murmur A somewhat accentu ated 3rd heart sound was noted in six

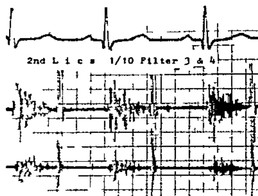


Fig 2 Decrescendo systolic murmur filling 80—90 of the S1—S2 interval

weaker transmission than the vibratory murmur (8) On phonocardiography its maximum often merges with the first sound with a gradual decrescendo in early or mid systole (fig 2) The murmur may also manifest a short crescendo to a peak a little after the first sound It has a slightly higher pitch and more blowing quality and shows somewhat more disturbance of the simple wave form than the vibratory murmur (9)

Intracardiac phonocardiography has shown the presence of a soft systolic ejection murmur in the main pulmonary artery in all normal individuals which explains the pulmonary systolic murmur (20) The vibratory murmur is also thought to be caused by vibrations of the pulmonary valve (13) Its low site of maximal intensity does not suggest a pulmonic origin however As right sided intracardiac phonocardiography fails to demonstrate this murmur it most probably has its origin in the left heart (25) This view is also supported by experiments with Valsalva maneuvers (14) where the vibratory murmur reappears gradually and attains a maximum 4—10 sec after breath release (4) the murmur of aortic stenosis in contrast with the immediate reappearance of the pulmonary ejection murmurs)

The term vibratory is not commonly used in our country in the description of murmurs We have classified them more objectively on the basis of the phonocardiograms Here we think that the crescendo decrescendo murmurs correspond to the vibratory type which then is the most common in our material as it was in other materials (4 14 15)

3 *Cardiorespiratory murmurs* and

4 *The supraclavicular arterial bruit* transmitted to the upper precordium were not encountered in our material of right heart catheterized patients

5 *The venous hum* was observed only once over the upper precordium In three other cases it was heard in the neck only

This is in contrast to the very frequent finding of venous and arterial neck bruits in children and young adults reported by other authors (5 6) One obvious reason for our infrequent finding of venous hum is that we have not auscultated the neck in sitting position as a routine procedure

The physiological murmurs are most often found in children the vibratory type being most prevalent at six years of age (4) and the pulmonic murmur being rare in this age group and more frequent in adolescence (4) Some of these murmurs seem to disappear before 10 years of age but most of them after puberty This may be partly due to the relatively

greater intensity of all heart sounds and also murmurs in children and adolescents, compared with adults In the report of Weaver and Walker (23) all patients retained their murmur, which, however, in 19 % was not present at rest, but only after exercise, on expiration, or in the standing position

Our material is not representative of the true age distribution of a population with physiological murmurs, as it only deals with patients on whom heart catheterization has been carried out It is, however, evident, that these murmurs also exist in the adult Eighty one % of the patients were in the age group 5—19 years, indicating that the physiological murmurs are most frequent in this age group One must, however also consider that this age group is most frequently submitted to health examinations In our material the different types of murmurs were not confined to any special age groups (table IV)

We excluded from the material pathologic conditions with increased cardiac output, e g thyrotoxicosis and anemia, where functional murmurs are often found

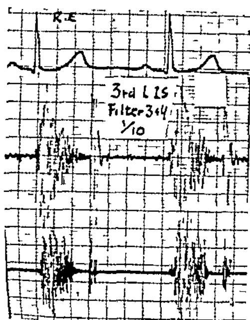


Fig 3 Systolic murmur of a small ventricular septal defect which with right heart catheterization was manifest only by positive hydrogen curves

Some organic disorders of the heart and great vessels may give rise to murmurs which may be difficult to separate from physiological ones Usually the former are louder, longer and of a more harsh quality — with a more complex composition consisting of high middle and low frequency components In *mitral incompetence* the murmur is usually holosystolic, extending to the axilla and back being weak upwards along the left sternal border — contrary to the physiological ones (16) In mild incompetence the murmur is sometimes only late systolic (1) The *ventricular septal defect* murmur is also usually holosystolic, but may in rare cases be confined to early systole (when a small defect in the muscular septum closes by contraction of the septum) On the phonocardiogram the murmur is often recorded with nearly the same width in the high and middle frequency filters and merges with the first sound covering this (fig 3) On the other hand a physiological murmur may be holosystolic although rather weak in late systole (7) and generally weak in the high frequency filter

In slight *pulmonic stenosis* P2 need not be markedly weaker than normal but usually it is delayed causing split

ting of the second sound, which, however, shows normal respiratory variations Leatham et al (12) state that a murmur raises the suspicion of *aortic disease* when it is grade 2/6 or more in the aortic area, is loud or louder in the mitral area, and less loud in the pulmonic and lower left sternal edge areas

The *atrial septal defect* murmur has very much in common with the physiological pulmonic ejection murmurs, the pathogenesis, localization and timing being much the same But the atrial septal defect murmur is usually louder and is accompanied by a constantly split second sound In slow and deep breathing, expiratory fusion of the two components of the second sound will almost invariably be obtained in normal subjects (12) Some rare cases may show constant splitting in supine position and a normal variation when sitting up A Valsalva maneuver may also be helpful fusion of A2—P2 will be achieved in the first 4—6 heart beats after termination of a 10 sec Valsalva maneuver (10) A constantly split second sound was a relatively frequent finding in our material This may be due to an inadequate respiratory effect on circulation in some cases Evaluation of the second sound in sitting position, and after Valsalva, has generally been done only in the later cases Accentuation of P2 was found fairly often in our cases In children and adolescents A2 often dominates the second sound in the pulmonic area To ascertain whether P2 is accentuated, one must therefore be careful to distinguish both components on auscultation and on the phonocardiogram

The electrocardiographic finding indicating diastolic overload of the right ventricle in 11 % of our material emphasizes the ambiguity of this sign, which may be found as a normal variant Moreover, the transition from slurring or notching of the QRS in V_1 to the frank signs of diastolic overload of the right ventricle is a gradual one

The great frequency and complexity of the possible positive heart X ray findings indicate the difficulty in drawing a sharp borderline between the normal and the pathologic In particular there seems to be a gradual transition from a somewhat prominent but not definitely abnormal pulmonary artery to an idiopathic dilatation of the pulmonary artery

Right heart catheterization with hydrogen studies should be fairly reliable in excluding right sided lesions and intracardiac shunts, but is uncertain in slight or moderate left sided lesions, such as aortic stenosis and mitral incompetence, where there is no pressure elevation in the pulmonary capillaries Here clinical and X ray findings are of greater importance, and left ventricular catheterization and angiocardiography are usually decisive Initial phases of primary myocardial diseases with septal hypertrophy and latent stenosis of left or right ventricular outflow tract may be impossible to diagnose on the first examination, although infusion with isoprenaline may give valuable information Several years follow up may be necessary There is however, the problem which of these cases should be advised to attend for regular check up in view of the great tendency to heart neurosis that such advice will entail

Summary

The report deals with 70 patients with systolic murmurs over the precordium finally judged to be physiological after complete cardiac evaluation including right heart catheterization (in 53 supported by hydrogen curves) The clinical doubt concerning the nature of the murmur was due partly to the character of the murmur itself or a split second sound partly to symptoms which might be referred to the circulatory system and to borderline X ray of the heart in some cases also to borderline ECGs Most of the subjects (81 %) were between 5 and 19 years old Nearly half of them had their murmurs discovered on routine medical check up Forty three % of the murmurs were below grade 3 6 The area of maximal intensity was in the 2nd left interspace in 54 % of the patients The murmurs were classified according to the shape and length on the phonocardiograms crescendo-decrescendo in 36 cases decrescendo in 16 a combination of the two in ten spool shaped holosystolic in five varying in two and continuous in one patient In 85 % the murmur occupied 60 % or more of the interval between the 1st and 2nd sound in the medium frequency filter recording where they were most dominant

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atropine for its elimination, but later no drugs were administered, because it disappeared spontaneously Their work however does not reveal how many of the patients with organic heart disease showed this arrhythmia They established broadly that of the 179 patients in the "heart disease group 51 4 % exhibited some form of arrhythmia, whereas only 19 8 % of the 390 "no heart disease" patients did so This arrhythmia has also been experimentally produced during halothane anaesthesia Purchase (8) in 1966 used an isolated cat heart perfused with a solution containing halothane (0 1 ml l) He established that halothane mainly affects atrioventricular conduction and that bradycardia will appear first and thereafter nodal rhythm This is followed by A—V dissociation with interference and then by A—V dissociation Finally asystole occurs This sequence of changes is also brought about in intact cats during halothane anaesthesia (9)

Methods

Most of the recordings were taken in the operating theatres during eye surgery Otherwise the patients were selected at random Pre-operative ECGs chest X ray and normal laboratory tests were done routinely whenever possible Blood pressure and pulse rate were recorded at 5 minute intervals throughout the operation and during the early post operative period The ECG was continuously monitored using ORM 2 and Videograph one-channel oscilloscopes and a Mingograph recorder Standard limb leads (leads I and II) were used ECG recordings were taken routinely on the operating table before anaesthesia during induction and intubation for general anaesthesia and during infiltration of local anaesthetic (2 % Xylocaine®), during surgical stimuli, and when indicated by changes seen on the monitoring oscilloscope screen As only the recordings were analysed there may in fact have been some more A V dissociations which were seen transiently but not recorded

Results

The series comprises 778 patients operated upon during halothane, local or epidural anaesthesia

Five hundred and eighty two patients were operated upon under halothane anaesthesia, of whom 362 were undergoing ocular operations

Halothane is a volatile anaesthetic, the major advantages of which are easy induction without irritation, rapid recovery, and, rarely, post operative nausea Increased vagal activity as well as hypotension is present during this type of anaesthesia (1)

Atrioventricular dissociation was present in 78 patients (13 4 %) at different stages of anaesthesia It was seen in 14 patients during intubation and reappeared later during operation Thus, among patients subjected to this form of anaesthesia the total number showing this arrhythmia was 92 (15 8 %) The greatest incidence of this arrhythmia was seen during ophthalmic operations, namely 86 atrioventricular dissociations in 72 patients There were 220 other surgical patients, among whom the arrhythmia was recorded in six

Table I shows the incidence of atrioventricular dissociation at different stages of halothane anaesthesia in the 78 patients

TABLE I Atrioventricular dissociation during halothane anaesthesia

Occurrence of A—V dissociation during	No of pats	Isorhythmic A—V dissociation	A—V dissociation with interference
Induction	164	2	—
Intubation with halothane	138	22	2
Intubation with halothane and succinylcholine	26	5	—
Intubation with thiopentone and succinylcholine	418	13	1
Maintenance of anaesthesia	582	7	2
Oculocardiac reflex	362	34	4
Total		83	9

As mentioned previously, all but six patients exhibiting A—V dissociation were ophthalmic cases The majority of these patients were children (2—19 years of age, 77 %), who were operated upon for strabismus Organic heart disease was not clinically established in any of these children In 164 cases the induction was performed with halothane and two patients under 10 years of age developed A—V dissociation during this procedure The reason for this arrhythmia was evidently the vagal effect caused by halothane as children usually have a pre existing vagal tone

The number of children under 15 years was 63, the mean age being a little under 8 years There were nine adults (20—77 years of age) Their mean age was 38 years

As can be seen from the table, atrioventricular dissociation occurred in 43 cases during intubation The patients were always ventilated with oxygen prior to this procedure When intubation was performed with halothane alone, this arrhythmia was present in 24 cases (17 4 %) (fig 1) All these cases were infants, induction and intubation of infants generally being carried out with halothane Their mean age was somewhat under 6 years In 14 cases during the ocular operation itself this arrhythmia was not seen In seven patients various vagal arrhythmias were seen such as wandering pacemaker, nodal rhythms, sinoatrial blocks, etc Four patients developed sinus tachycardia during the operation, a sign of the sympathetic component of this reflex arc on the other hand, three patients showed no arrhythmias during the operation Two out of these 14 patients developed *dissociation with interference* during intubation Neither of them had organic heart disease The arrhythmia disappeared when atropine was given intravenously These two patients during the operation for strabismus further developed either nodal rhythm or sinoatrial block and nodal escape beats, as a consequence of the vagal irritation caused

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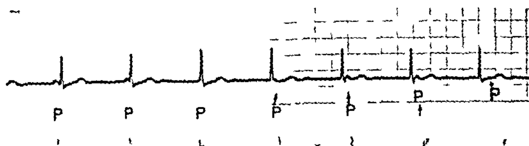


Fig 1 Isorhythmic A—V dissociation in a girl aged six during intubation with halothane (lead II)

by the oculocardiac reflex in spite of atropine administration in connection with the arrhythmia occurring during intubation Two patients who developed isorhythmic A V dissociation were given atropine and the arrhythmia immediately disappeared In other patients this arrhythmia disappeared during oxygen ventilation after intubation During halothane intubation ten patients developed isorhythmic A—V dissociation which however disappeared with deep anaesthesia and elimination of irritation caused by intubation It reappeared provoked by a strong vasovagal reflex when the eye muscles were stretched during operation When this occurred there was an interval of normal sinus rhythm sometimes lasting about one minute between the two periods of A—V dissociation In two out of these ten cases atropine was given because of the A V dissociation appearing during intubation yet it reappeared during the operation for strabismus being discharged by the strong oculocardiac reflex

Intubation with halothane and succinylcholine was performed in 26 patients All these were children under ten years of age In five cases atrioventricular dissociation (19 2 %) was present Succinylcholine, which is a muscle relaxant, has a vagal effect similar to that of halothane In three cases atropine was administered and this arrhythmia immediately disappeared and in two other cases it disappeared by itself somewhat later

The 418 patients of the series intubated with thiopentone and succinylcholine consisted of older children and adults Atrioventricular dissociation was present in 14 cases (3 3 %) Of these cases ten developed the dissociation only during intubation and four during both intubation and the operations Of the patients developing it only during intubation six were children and four were adults (range 3—57 years of age) As is seen from the table, there was one dissociation with interference This arrhythmia developed in a woman aged 49 who underwent cholecystectomy The X ray showed an enlarged heart and slight changes were seen in the ECG Obviously this arrhythmia was caused by transient hypoxia during intubation and by the vagal effect of succinylcholine together with the vasovagal reflex provoked by intubation It should be noted that this instance of A—V dissociation

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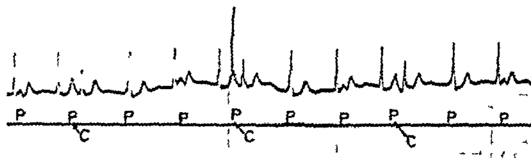


Fig 2 A V dissociation with interference in a man aged 57 during thoracotomy (arrows con ducted beats) (lead II

2 7 % (the sixth patient of the group developed dissociation with interference during intubation as described previous ly The ages of these patients ranged from 33 to 67 years Four of them were subjected to abdominal operations and one patient underwent thoracotomy for a suspected thoracic tumour The latter patient developed A—V dissociation with interference when the hilum of the lung was stretched (fig 2) No greater electrocardiographic changes were re corded prior to operation The arrhyth mia disappeared later when the ventila tion was improved One of the abdomi nal cases underwent gastroenterostomy for inoperable carcinoma of the stomach He had coronary heart disease and had taken digitalis for a long period The blood pressure fell during operation to 80 mm Hg and dissociation with inter ference developed the coronary blood flow being further reduced The disso ciation disappeared later after oxygena tion and administration of metaraminol and when the blood pressure had risen to 130 mm Hg Three other patients developed isorhythmic A V dissocia tion during intra abdominal surgery These patients showed no organic heart disease, and oxygenation eliminated the arrhythmia In all these patients serum electrolytes, serum proteins, serum creatinine and acid base balance were within normal limits

Four other patients who developed A—V dissociation during maintenance of anaesthesia were young ocular cases in whom halothane evidently caused this arrhythmia as a consequence of in creased vagal tone already present be fore the beginning of the operation

During operations carried out under local anaesthesia A—V dissociation was present in two patients and A—V disso ciation with interference in one Al together 111 patients were operated upon under this form of anaesthesia and there were 103 ocular and eight other kinds of operations The three patients mentioned here were ocular cases operated upon for strabismus and the arrhythmia developed during stretching of the eye muscles initiated by the oculocardiac reflex The patients were males (aged 15 23 and 15 years) No organic heart disease could be estab lished in these patients The arrhythmia disappeared after termination of the reflex irritation All the patients were

premedicated with atropine, which, however did not inhibit the development of the arrhythmia

Elderly patients were usually operated upon under epidural anaesthesia, and they underwent some major surgical (principally prostatectomy) or gynaecological operation The anaesthetic agent used was prilocaine 1 5 % Metaraminol was always administered prior to operation In spite of this there was often a fall of blood pressure following the spread of analgesia The series comprised 85 patients Organic heart diseases were more common than in the other groups (viz 15 patients) Four patients developed isorhythmic A—V dissociation (4 7 %) Two of them did not have organic heart disease Isorhythmic A—V dissociation was always present with marked reduction of blood pressure After readministration of metaraminol to three patients the arrhythmia disappeared when the blood pressure had increased In the fourth patient the arrhythmia did not disappear until the following day, although the blood pressure had reached its former level and the ST changes caused by hypotension were corrected immediately after the administration of metaraminol

Discussion

There were a number of A—V dissociations in this series during halothane anaesthesia as is evident from the above The observations made by Dodd et al (2) and Silverblatt et al (14) also revealed a high incidence of this arrhythmia Their results, however, are not comparable, more of their patients being in the older age groups, the majority of whom had organic heart disease and among whom there were also poor risk patients The anaesthetic agents used for general anaesthesia were different and ophthalmic cases were not included in their series

On the other hand in this series there were surprisingly few patients with electrocardiographic signs of coronary heart disease (only nine patients out of 220 surgical patients and three out of 362 ophthalmic patients) Such disease therefore has little importance in the origin of this arrhythmia Furthermore, half the present series consisted of young eye patients with a normal heart In the majority of cases it was considered that when during halothane anaesthesia, this arrhythmia developed it was discharged by the vasovagal reflex But in three instances, in patients having coronary heart disease the arrhythmia was an obvious consequence of the deterioration of the circulation to ischaemic heart muscle All these arrhythmias were dissociations with interference It is well known that the aetiology of dissociation with interference in most instances is connected with pre existing organic heart disease

About the half of the A—V dissociations of this series appeared during intubation It is recognized that intubation causes an intense reflex irritation The arrhythmia was most frequent in those cases where halothane (17 4 %) or halothane and succinylcholine (19 4 %) were used All these patients were children who as is well known, have increased vagal tone This together with the central vagal effect of halothane

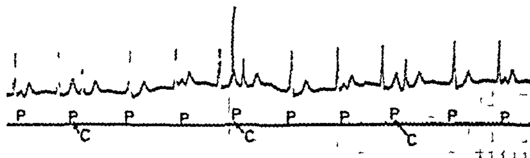


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During operations carried out under local anaesthesia A—V dissociation was present in two patients and A—V dissociation with interference in one. Altogether 111 patients were operated upon under this form of anaesthesia and there were 103 ocular and eight other kinds of operations. The three patients mentioned here were ocular cases operated upon for strabismus, and the arrhythmia developed during stretching of the eye muscles initiated by the oculocardiac reflex. The patients were males (aged 15, 23 and 45 years). No organic heart disease could be established in these patients. The arrhythmia disappeared after termination of the reflex irritation. All the patients were

premedicated with atropine, which, however, did not inhibit the development of the arrhythmia

Elderly patients were usually operated upon under epidural anaesthesia, and they underwent some major surgical (principally prostatectomy) or gynaecological operation The anaesthetic agent used was prilocaine 1 5 % Metaraminol was always administered prior to operation In spite of this there was often a fall of blood pressure following the spread of analgesia The series comprised 85 patients Organic heart diseases were more common than in the other groups (viz 15 patients) Four patients developed isorhythmic A—V dissociation (4 7 %) Two of them did not have organic heart disease Isorhythmic A—V dissociation was always present with marked reduction of blood pressure After readministration of metaraminol to three patients the arrhythmia disappeared, when the blood pressure had increased In the fourth patient the arrhythmia did not disappear until the following day, although the blood pressure had reached its former level and the ST changes caused by hypotension were corrected immediately after the administration of metaraminol

Discussion

There were a number of A—V dissociations in this series during halothane anaesthesia, as is evident from the above The observations made by Dodd et al (2) and Silverblatt et al (14) also revealed a high incidence of this arrhythmia Their results, however are not comparable more of their patients belong to the older age [illegible] majority of whom had [illegible] disease and among whom [illegible] also poor-risk patients The [illegible] agents used for general anaesthesia [illegible] different and ophthalmic cases were [illegible] included in their series

On the other hand in the [illegible] were [illegible] only few patients [illegible] electrocardiographic signs of coronary disease (only nine patients [illegible] surgical patients and three [illegible] ophthalmic patients) Such [illegible] therefore has little importance [illegible] origin of this arrhythmia Furthermore half the present series consisted of [illegible] eye patients with a normal heart In [illegible] majority of cases it was considered [illegible] when, during halothane anaesthesia this arrhythmia developed it was discharged by the vasovagal reflex but in three instances, in patients having coronary heart disease, the arrhythmia was [illegible] obvious consequence of the deterioration of the circulation to ischaemic heart muscle All these arrhythmias were dissociations with interference It is well known that the aetiology of dissociation with interference in most instances is connected with pre-existing organic heart disease

About the half of the A—V dissociations of this series appeared during intubation It is recognized that intubation causes an intense reflex irritation The arrhythmia was most frequent in those cases where halothane (17 4 %) or halothane and succinylcholine (19 4 %) were used All these patients were children who as is well known have increased vagal tone This together with the central vagal effect of halothane,

potentiates the vasovagal reflex caused by this procedure. Illes and Defensar (5) have emphasized that succinylcholine when used to facilitate intubation in children increases the frequency of various arrhythmias. In this series these two groups of patients were so unequal numerically that no conclusions can be drawn.

All the adults and older children were intubated with thiopentone and succinylcholine. Atrioventricular dissociation was present in only 3.3 %. This could be due to the fact that the central vagal effect of succinylcholine is not so strong as that of halothane. The mean age of the patients in this group was higher and correspondingly the vagal tone more reduced.

The incidence of a positive oculocardiac reflex particularly in relation to the onset of this arrhythmia was rather high. It is one of the strongest vasovagal reflexes and is easily discharged by for example stretching of the ocular muscles or by bulbar pressure. It is considered positive if the heart rate slows down by at least 10 beats/min. or if various arrhythmias appear (10, 13). In the nerve pathways of this reflex there are mainly vagal but also some sympathetic nerve fibres (7). Therefore tachycardia may be present as a consequence (inverted oculocardiac reflex). This reflex is seen less in adults than in children and has been found to be positive in children under 15 years in as many as 90 % of cases (12). In four instances dissociation with interference was observed. In two cases in children with a normal heart it was considered to be due to this reflex. On the other hand, a man aged 77 with coronary heart disease developed intense hypotension during anaesthesia, so that the slowed coronary circulation caused by it may be regarded as an important aetiological factor. A woman aged 21 with a healthy heart also developed intense hypotension during an operation for strabismus, which in this case may be considered a possible additional factor in the origin of the interference. The termination of reflex irritation was generally sufficient to eliminate the atrioventricular dissociation occurring during the oculocardiac reflex. In some cases atropine was given, thereby correcting the dissociation.

During maintenance of anaesthesia there were two dissociations with interference, one of which appeared during thoracotomy on stretching the lung hilum — the obvious cause being the strong vasovagal reflex caused by this manipulation. An additional cause could have been momentary hypoxia that caused changes in the metabolism of the myocardium, thereby increasing the effect of hypercapnia and thus causing vagal irritation (4). Another arrhythmia of this kind developed in a patient with coronary heart disease. Evidently in this case the insult of hypoxia combined with anaesthesia had a deleterious effect on the patient's already ischaemic myocardium (3).

Accordingly it is considered that the occurrence of atrioventricular dissociation during operations under halothane anaesthesia is harmless and transient. A pre-medicative dose of atropine was given routinely prior to anaesthesia (11). In spite of this measure this arrhythmia did appear, the cause apparently being

a cessation of the atropine effect during operation Oxygenation often terminated the arrhythmia, but if this was not successful atropine was readministered intravenously and the arrhythmia was terminated A repeated dose of atropine given immediately prior to the ocular operation did not inhibit the onset of atrioventricular dissociation which is a proof of the strong effect of the oculocardiac reflex

Only three patients showed this arrhythmia during local anaesthesia (2 7 %), and it is regarded as being caused by the oculocardiac reflex in spite of the fact that analgesia was considered satisfactory

During epidural anaesthesia A—V dissociation was observed in four patients (4 7 %), and it always appeared during marked reduction in blood pressure and as an evident consequence of a hypoxic myocardium

Summary

The eries comprises 778 patients operated upon under halothane, local or epidural anaesthesia Only a few patients had organic heart disease The number of patients operated upon under halothane anaesthesia was 582, of whom 362 had ocular surgery A—V dissociation was seen during this form of anaesthesia in 78 patients (13 4 %) In 14 patients it was present during intubation and reappeared later during operation The majority of the arrhythmias occurred during ocular operations viz in 72 patients In other surgical patients it was recorded only six times There were 83 patients with isorhythmic dissociation and nine with dissociation with interference During intubation the arrhythmia was present in 43 patients In 38 patients (10 5 %) the arrhythmia was regarded as being cau ed by the oculocardiac reflex

A—V dissociation occurred in nine patients during maintenance of halothane anaesthesia Five of them underwent some operation other than ocular

Three patients out of 111 subjected to local anaesthesia developed A—V dissociation They were ophthalmic cases

During operations performed under epidural anaesthesia this arrhythmia occurred in four out of 85 patients and it was always seen concomitantly with a sudden fall of blood pressure

In the majority of cases it was regarded as being due to the vasovagal reflex potentiated by increased vagal tone caused by halothane In only three cases of dissociation with interference was apparently a matter of reduced blood flow to the ischaemic myocardium of patients suffering from coronary heart disease

The occurrence of A—V dissociation in the present series has been regarded as harmless and transient and it has been corrected with oxygenation or atropine or through elevation of the blood pressure during epidural anaesthesia

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Chromosome Studies in Acute Leukaemia

II A comparison between the chromosome patterns of bone marrow cells and cells from the peripheral blood

By

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During the last few years the chromosome patterns of bone marrow and blood cells in acute leukaemia have been intensively studied Normal as well as abnormal findings have been reported In 1962 Sandberg et al (13) drew attention to the inadequacy of the blood culture method as regards detection of abnormal karyotypes in acute leukaemia In fact, few chromosome abnormalities have been found in studies dealing only with blood cultures (4, 12, 16) It should, however, be recognized that most data on the chromosome constitution of the blood cells of leukaemic patients — including those of Sandberg et al (13) — are based on studies of blood cultures with phytohaemagglutinin (PHA) added (4 5 12 16) It is known that PHA stimulates lymphocytes of normal blood to proliferate (1 9) When leukaemic blood is cultured with PHA, mitotic figure may be derived from two sources leukaemic cells and PHA stimulated lymphocytes The lack of abnormal karyotypes in PHA cultured leukaemic blood might be due simply to a preponderance of mitotic figures derived from stimulated lymphoid cells It was therefore decided to compare the chromosomal findings in bone marrow aspirates with those in the peripheral blood cells cultured both with and without PHA in a group of patients with acute leukaemia

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Material and methods

During the past two years marrow aspirates from 28 patients with acute leukaemia were studied cytogenetically Attempts to culture the peripheral blood both with and without PHA were made in 14 of these patients during relapse and were successful in ten cases Only these ten patients are included in the present report.

The series includes five men and five women ranging in age from 27 to 75 years

TABLE I Cytological type of leukaemia and therapy of the patients

Patient no	Sex	Age (yrs)	Type of leukaemia	Chemotherapy prior to study of	
				Marrow cells	Cultured blood cells
1		75	Acute erythroleuk	None	None
2		49	Myeloblastic	None	None
3		62	Acute erythroleuk	None	None
4	♂	56	Myeloblastic	None	None
5	♂	51	Monocytic	None	None
6	♂	74	Myeloblastic	None	None
7		68	Myeloblastic	None	None
8	♀	27	Lymphoblastic	6-MP[1] 11 months	Methotrexate 5 weeks
9		70	Myeloblastic	None	6-MP[1] 3 weeks
10	♂	35	Promyelocytic	None	None

1 MP 6 mercaptopurine

Data regarding therapy and the cytological type of leukaemia are presented in table I For cytogenetic observations the bone marrow aspirates were treated according to a slight modification of the technique described by Tjio and Whang (15 without prior in vitro culture Blood cultures were prepared by a slight modification of the method of Moorhead et al 10 Thus from each patient cultures of the peripheral blood were set up both with and without PHA The former cultures were harvested after 72 hours the latter after 24 or 48 hours of culture Four hours before termination Colcemid® 0 5 ml of a 0 01 % solution was added to the cultures each having a volume of 5 ml

Results

Table II depicts the chromosomal findings in the bone marrow aspirates and the blood cultures of the patients

In three of the patients viz nos 3 9 and 10 no cells in mitosis were present in the cultures set up without PHA In patients nos 7 and 8 the blood cultures without PHA yielded only a few scoreable metaphases

The marrow aspirates of three patients (nos 3 4 and 7) had normal diploid modes No abnormal cell line was present In the same patients both types of blood cultures had normal chromosomal patterns without any abnormal cell line

In the remaining seven patients an abnormal cell line was present in the bone marrow A brief survey of the chromosomal findings in these patients is given

Figures of aneuploid metaphases from patients nos 1 9 and 10 have previously been published (7 8)

Case reports

Case 1

A marrow aspirate obtained at the time of admission had a mode of 42 chromosomes

TABLE II Chromosomal findings in bone marrow aspirates and peripheral blood cells in ten cases of acute leukaemia

Pat no	Date	Type of tissue	Total cells scored	Chromosome number										No of cells with marker chromosomes
				<40	40	41	42	43	44	45	46	47	>47	
1	10 5 65	Marrow	50	2		6	36	3	1	1	1			36
	29 5 65	Blood[1] without PHA	21			1	18				2			13
	29 5 65	Blood with PHA	50	1	2	1	1	2		4	37	1	1	
	31 7 65	Marrow	50	2	3	1	39	4	1					44
	31 7 65	Blood[1] without PHA	50	5	3	6	31	5						37
	31 7 65	Blood with PHA	50			2	23	2	1	1	21			22
2	3 6 65	Marrow	50						4	38	8			
	3 6 65	Blood[1] without PHA	15						1	8	6			
	3 6 65	Blood with PHA	50							4	45		1	
3	23 6 65	Marrow	50				1			3	45		1	
	23 6 65	Blood with PHA	50						1	1	47		1	
4	25 6 65	Marrow	50			2		1	4	3	40			
	25 6 65	Blood[1] without PHA	50	1			3	2	1	11	32			
	25 6 65	Blood with PHA	50				1	2	2	4	40	1		
5	30 6 65	Marrow	50	2	1	1		1	2	41	2			
	30 6 65	Blood[1] without PHA	50			1			1	5	42	1		
	30 6 65	Blood with PHA	50			1				5	40	4		

(Cont)

Table II Cont

Pat no	Date	Type of tissue	Total cells scored	40	40	41	42	43	44	45	46	47	>47	No of cells with marker chromosomes
C	2 10 65	Marrow	50						4	6	31	9		
	? 10 65	Blood without PHA	0							5	22	?3		
	? 10 6	Blood with PHA	0				1		?	5	41	1		
	1	Marrow	0					1	2	4	43			
		Blood without PHA	5								5			
	11 6	Blood with PHA	?0					1	1	1	17			
8	1 12 65	Marrow	50							1	18	31		
	8 ? (	Blood without PHA	9									9		
	8 66	Blood with PHA	50				1	(		3	4?			
9	3 1? 6	Marrow	44	2	4	?1	9	?	1		?			32
	1 1 66	Blood with PHA	50					1	3	6	40			
10	(66	Marrow	50	1		2		3	42	2				41
	?1 ? 66	Blood with PHA	0							6	43	1		

Two-day culture
* One-day culture
PHA phytohaemagglutinin

Seventy two % of the metaphases contained a ring chromosome. A blood culture set up without PHA 1? days later had the same mode of 4? chromosomes. In 6? % of the metaphases the ring chromosome was present. A blood culture with PHA added had a normal diploid mode and the marker chromosome was absent. A second marrow aspirate obtained ? 1/? months later again showed the same abnormal mode with a ring

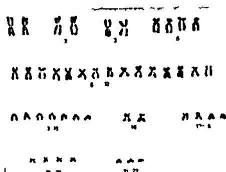


Fig 1 Marrow metaphase from patient no 2 containing 45 chromosomes. One chromosome is missing in the groups 6 X 12 and 21 22 A supernumerary chromosome is present in group 13 15

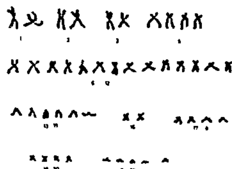


Fig 2 Marrow metaphase from patient no 5 containing 45 chromosomes One chromosome is missing in group 6 X 12



Fig 3 Blood metaphase from patient no 6 containing 47 chromosomes A supernumerary chromosome is present in group 6 X 12

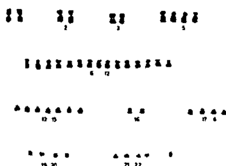


Fig 4 Marrow metaphase from patient no 8 containing 47 chromosomes A supernumerary chromosome is present in group 13 15

chomosome present in 88 % of the meta phases At the same time a blood culture set up without PHA had a similar mode and 74 % of the metaphases contained a ring chromosome A blood specimen cultured with PHA showed a bimodal distribution of chromosome numbers 46 % of the meta phases contained 42 chromosomes and 42 % were diploid In most of the hypodiploid metaphases a ring chromosome was present

Case 2

About 80 % of marrow metaphases had a mode of 45 chromosomes Karyotype analysis in ten cells revealed that the hypodiploidy was due to absence of a chromosome in the groups 6 X 12 and 21 22 and a super numerary chromosome in group 13 15 (fig 1) In a blood culture without PHA only 15 metaphases could be scored eight of which contained 45 chromosomes Six of these hypodiploid cells had the same abnor mal karyotype as was present in the bone marrow A blood culture set up with PHA had the normal diploid mode

Case 5

Ninety % of the marrow metaphases had 45 chromo omes Karyotype analysis was per formed in 16 of the hypodiploid metaphases

in all of them a chromosome of group 6-X 12 was missing (fig 2) Blood cultures with and without PHA added had normal diploid modes In the blood culture without PHA added five cells contained 45 chromosomes in three of which a chromosome of group 6-X 12 was missing

Case 6

The marrow cells had a mode of 46 chromosomes About 20 % of the metaphases contained 47 chromosomes Karyotype analysis in four of these metaphases revealed that the hyperdiploidy was due to an extra chromosome belonging to group 6-X 12 At the same time about 50 % of the metaphases of a blood culture set up without PHA contained 47 chromosomes (fig 3), whereas a blood specimen cultured with PHA had a normal diploid mode

Case 8

The marrow mitoses had a mode of 47 chromosomes Few hyperdiploid metaphases could be karyotyped In these an extra acrocentric chromosome with the size of the chromosomes of group 13 15 was seen (fig 4) In a blood specimen cultured without PHA about two months later only nine metaphases could be scored all of which had 47 chromosomes A blood culture with PHA added had a normal diploid mode

Case 9

Only two cells with a normal female karyotype were encountered in the marrow aspirate About 50 % of the metaphases contained 41 chromosomes About 75 % of the marrow metaphases contained a marker chromosome i.e. a large acrocentric chromosome A blood culture set up three weeks later with PHA added had a normal female karyotype No marker chromosomes were present A blood specimen cultured without PHA yielded no mitotic figures

Case 10

In the marrow aspirate about 80 % of the metaphases contained 44 chromosomes In about 80 % of the metaphases one of the chromosomes belonging to group 21 22 Y was considerably smaller than the other chromosomes of this group, possibly due to a deletion of the long arms A blood culture without PHA yielded no mitotic figures The peripheral blood cells cultured with PHA had a normal diploid mode No abnormal chromosomes were seen

Discussion

When the peripheral blood cells from normal individuals are cultured in vitro few if any of the cells are capable of dividing However, when PHA — an extract of the red kidney bean — is added to the culture medium, large primitive "blast like" cells appear which are capable of undergoing mitosis (11) These cells are known to be of lymphocytic origin (1, 9) About 20 hours after the addition of PHA to the culture the first mitotic figures are encountered A peak of mitosis is seen from about 55 to 72 hours after initiation of the culture (2)

In contrast, the immature cells of the peripheral blood from patients with acute leukaemia are able to proliferate without the addition of a mitogenic substance to the medium Thus, Elves and Wilkinson (2) found a mitotic peak during the first few hours after initiation of culture of the peripheral blood from patients with acute leukaemia The number of dividing cells then rapidly decreased and after 30 hours mitotic figures were no longer present When PHA was added to the cultures, another mitotic peak was seen about 40 hours later This finding was considered to be due to proliferation of normal lymphocytes present in the leukaemic blood

In view of these proliferative characteristics of normal lymphocytes and

leukaemic cells, it is to be expected that after 72 hours culture of leukaemic blood with PHA there will be found mainly — or exclusively — lymphocytic mitotic figures The results of the present study are in accordance with this expectation Thus, normal diploid modes were found in the PHA blood cultures from the seven patients in whom aneuploid cell lines were present in the marrow Only one PHA culture (case 1) was bimodal, probably due to the presence of both proliferating leukaemic cells and normal lymphocytes These findings explain why so few chromosome abnormalities have been demonstrated in several series of patients with acute leukaemia, in these studies, only blood cultures with PHA added were employed for cytogenetic analysis (4, 12, 16)

The results of the chromosome studies performed on peripheral blood cells cultured for 24 –48 hours without PHA indicate that, quite frequently, the abnormal karyotypes which are present in the marrow can be demonstrated in the blood This is in agreement with the results of Hungerford and Nowell (6) and Fitzgerald et al (3) However, in some respects the study of cultured blood cells is less satisfactory than the study of marrow cells Thus, the cells may be incapable of proliferation and yield no mitotic figures as in cases 3, 9, and 10 of the present series Moreover, the proliferating cells in the cultures may be diploid although an aneuploid stem line is present in the bone marrow, as exemplified by case 5

On the other hand it is possible that aneuploid cells in some cases may have a proliferative advantage under the condition of culture as compared to the in vivo conditions in the bone marrow Thus, in patient no 6 of the present series the percentage of aneuploid cells in the blood cultures by far exceeded that of aneuploid cells in the bone marrow preparations Therefore, there is a possibility that in some cases of acute leukaemia an aneuploid cell line which cannot be demonstrated in the marrow aspirate may be detected in a blood specimen cultured without PHA This, however remains to be demonstrated

The presence of normal diploid metaphases in blood cultured without PHA in patients nos 2 and 5 who had an aneuploid mode in the marrow cells is a puzzling finding From where do these cells originate? There are several possibilities

1 The diploid metaphases may represent dividing normal erythroid or myeloid precursors which circulate in the peripheral blood

2 They may represent a clone of leukaemic cells with a normal diploid mode which exists pari passu with the aneuploid cells

3 They may represent a population of normal lymphocytes which exists in the leukaemic individual but which in contrast to the lymphocytes of normal individuals may be able to proliferate in vitro without the addition of a mitogenic agent One may consider the possibility that the lymphocytes may have responded to an antigen associated with the leukaemic cells (host versus graft reaction?)

The first possibility seems unlikely No circulating erythroid precursors or morphologically normal granulocytic

precursors could be demonstrated in the peripheral blood of patients nos 2 and 5 In addition, even if small numbers of erythroid precursors could have remained undetected this could hardly explain the presence of normal karyotypes in the blood, since highly suggestive evidence has recently been offered that chromosome abnormalities in acute leukaemia are present not only in the leukaemic blast cells but also in the erythroid precursors (8)

Neither of the remaining possibilities can be completely dismissed The second possibility implies that, in essence there are two different clones of leukaemic cells an aneuploid and a diploid cell line In patient no 5 in whom diploid metaphases were completely dominating in the blood culture without PHA, in contrast with the nearly uniform population of hypodiploid metaphases in the bone marrow, the diploid cell line should then have achieved a proliferative advantage during the in vitro conditions in the blood culture However a verification whether both karyotypes represented leukaemic blast cells would call for cytological identification of the metaphase figures which is not possible with present techniques

A few recent studies suggest that at least some of the dividing diploid cells in leukaemic blood cultured without PHA may originate from normal lymphocytes Thus Wills and Gross (17) found that normal lymphocytes cultured in plasma from patients with acute leukaemia or chronic myelocytic leukaemia in relapse showed considerable mitotic activity As regards chronic myelocytic leukaemia similar results have been reported by Sandberg et al (14) These observations suggest the presence in leukaemic plasma of mitogenic factors acting on lymphocytes It is interesting to speculate that the normal lymphocytes might proliferate in response to an antigen which is related to the leukaemic disease

The normal karyotypes present in PHA cultures of peripheral blood from leukaemic patients with aneuploid modes of the marrow cells indicate that the lymphocytes of the peripheral blood which are triggered into proliferation by PHA are unrelated to the leukaemic cells The fact that this also applies to lymphoblastic leukaemia, as demonstrated in case 8 of the present series, suggest that two types of "lymphocytic" cell lines may exist in this disorder, viz 'leukaemic lymphoblasts' which are present both in the bone marrow and in the peripheral blood and are able to enter mitosis without the addition of PHA, and normal lymphocytes which proliferate only when a mitogenic factor is added This lends further support to current concepts of the heterogeneous origin of lymphoid cells

Summary and conclusion

The bone marrow aspirates and blood cultures of ten patients with acute leukaemia were cytogenetically investigated In seven patients the marrow aspirates had aneuploid cell lines Blood from these patients cultured with PHA added had normal diploid modes with the exception of one culture which was bimodal In five cases, scoreable metaphases were obtained in blood cultured

for 24—48 hours without PHA Four cultures contained the same aneuploid cell line as was present in the bone marrow One culture, however, had a normal diploid mode although the marrow cells were aneuploid

It is concluded that blood cultured with PHA is inadequate for detecting the abnormal karyotypes which are present in bone marrow cells in acute leukaemia In contrast, when blood is cultured without PHA, abnormal karyotypes of the type present in the bone marrow can in some cases be detected This method is of particular value in patients in whom bone marrow cannot be aspirated

The present material included one patient with lymphoblastic leukaemia The lymphocytes of the peripheral blood cultured with PHA were diploid whereas mitoses in the bone marrow, which was completely dominated by lymphoblasts, were aneuploid The significance of this finding is discussed

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In Vitro Detection of Cellular Hypersensitivity in Man

Specific migration inhibition of white blood cells from brucella positive persons

By

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The role of cellular hypersensitivity in several immunological phenomena has attracted much attention during recent years The investigation of this type of specifically altered reactivity in man has depended mainly on the intracutaneous reaction and more recently on the skin window technique (24, 28)

In a previous paper (26) some of the practical and theoretical disadvantages of the methods have been discussed Besides an influence of circulating antibodies cannot be excluded either with the intracutaneous reaction or with the skin window technique

Methods for detection of cellular hypersensitivity in vitro have been in use for several years mainly in animal experiments

These methods entail recording the specific action of the antigen upon sensitized lymphocytes or other immuno competent cells Two approaches have been based on this principle a) blast cell transformation, and b) migration inhibition of immuno competent cells

The blast cell transformation test has been studied in man with various antigens (2, 8, 23) The results have been inconsistent, and the applicability of the method as a specific parameter of cellular hypersensitivity has been questioned on theoretical grounds (7)

With a single exception (21) the studies based upon specific inhibition of the migration of immuno-competent cells have been done only in animals The experiments done with this technique seem to indicate that the specific inhibition of migration is a reliable in vitro parameter of cellular hypersensitivity in animals (5 11 14, 25, 27)

Among other antigens brucella bacteria and brucellergen have been used in experiments with guinea pigs (4 10 15 16) With spleen cells macrophages and leucocytes from infected animals a distinct inhibition of cell migration was ob

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value of the brucella negative observations is 0.92 ± 0.07, while the mean value of the brucella positive observations is 0.58 ± 0.15 which implies that the two groups of observations are significantly different ($p < 0.001$)

Brucella antigen in the concentration applied (50 mill bact per ml) appears to be slightly toxic for the normal cells but in no case is the migration inhibition as marked as when the cells come from brucella positive individuals This implies that a migration inhibition of the hypersensitive white cells in the presence of brucella bacteria corresponds well with a state of brucella hypersensitivity as expressed in a positive cutaneous reaction

The lowest value of the migration inhibition in brucella negative persons in this material is 0.78 This seems to be the borderline between brucella negative and brucella positive observations but in larger materials a slight overlapping may be expected

In order to show clearly the relationship between the cutaneous reaction and

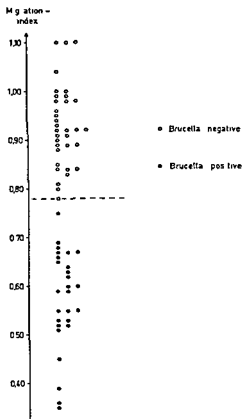


Fig 1 Migration indices of brucella negative and brucella positive persons

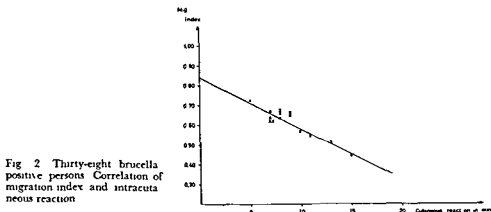


Fig 2 Thirty-eight brucella positive persons Correlation of migration index and intracutaneous reaction

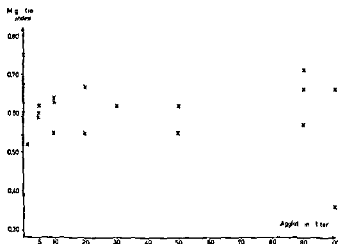


Fig 3 Thirty-eight brucella positive persons Correlation of migration index and agglutinin titer

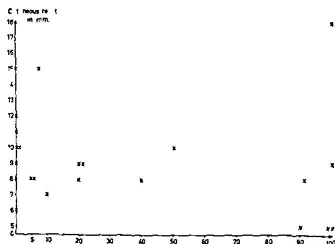


Fig 4 Thirty-eight brucella positive persons Correlation of intracutaneous reaction and agglutinin titer

the migration index, these two parameters have been plotted into a diagram (fig 2) It appears very clearly that there is a good correlation of the intracutaneous reaction and the migration index (r = — 0 88) The standard deviation of the migration indices around this regression line is 0 07 in the brucella positive persons *This is not significantly* different from that of the brucella negative observations (0 08)

Figs 3 and 4 show the correlation of agglutination titer and migration index and of agglutination titer and the cutaneous reaction in brucella positive persons It appears that there is no correlation of these parameters (r = 0 08 and r = 0 14)

Discussion

The specific inhibition of the migration of peripheral white blood cells from brucella positive persons is in agreement *with the results obtained in animal ex*periments with brucella antigen (4, 10

15 16) and other antigens inducing cellular hypersensitivity In the present work, however, to attain a constant migration inhibition it was necessary to apply a higher concentration of antigen than in the animal experiments

While the antigen concentration in the experiments with guinea pigs was only about 1 mill bact per ml (16) the concentration in the present experiments is as high as 50 mill bact per ml

This difference may be explained in various ways

1 The degree of hypersensitivity in the animal experiments is more pronounced, whereas in the experiments here described only a single antigen dose was given The immuno-competent cells from the animals might thus be sensitized to a higher degree

2 Cells from the spleen have been applied in some instances (15, 16), where they reacted more strongly to the antigen than for instance, leucocytes, this indicates that cells from the peripheral blood possess a lower degree of immuno-competence

3 Brucella Suis was used as an antigen in some of the animal experiments, accordingly a difference in antigenicity cannot be excluded

In the present experiments, the high concentration of antigen appears to inhibit only slightly the migration of normal cells The migration inhibition of the hypersensitive cells can thus be considered a specific phenomenon

The assumption that this in vitro technique is in fact giving a measure of cellular hypersensitivity is very strongly supported by the good correlation found between the migration inhibition and the cutaneous reaction Moreover, the lack of correlation between the agglutinating antibodies and the migration inhibition indicates that the latter is unrelated to the humoral sensitivity Thus a probable contamination of the cells by circulating antibodies does not influence the specificity of the reaction The same observation was made by David et al (11) In their experiments with guinea pigs, which were in a state of humoral hypersensitivity they could not elicit specific inhibition of the migration of immuno-competent cells

The mechanism of the specific migration inhibition is still badly understood Experiments by Bloom and Bennett (3) and David (12) seem to indicate that under the influence of the antigen, the sensitized cells elaborate a substance which appears in the medium and which can inhibit the migration of normal non sensitized cells This substance has been found present in the supernatant from sensitized cells cultured for 24 hours with the specific antigen

David used two types of cells sensitive lymphoid cells obtained from lymph nodes and peritoneal cells He found that the sensitive lymphoid cells themselves were not inhibited by specific antigen but that inhibition was seen with a mixed population of sensitive lymphocytes and normal peritoneal cells These findings suggest that two cell types are important in these reactions sensitive lymphocytes which elaborate a specific substance and cells from the peritoneal exudate presumably macrophages which need not to be sensitive As already mentioned the cells used in the present experiments consist of a mixture of approximately

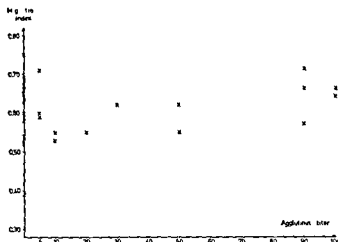


Fig 3 Thirty-eight brucella positive persons Correlation of migration index and agglutinin titer

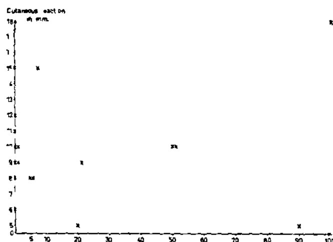


Fig 4 Thirty-eight brucella positive persons Correlation of intracutaneous reaction and agglutinin titer

the migration index these two parameters have been plotted into a diagram (fig 2) It appears very clearly that there is a good correlation of the intracutaneous reaction and the migration index (r = — 0 88) The standard deviation of the migration indices around this regression line is 0 07 in the brucella-positive persons This is not significantly different from that of the brucella negative observations (0 08)

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50 % lymphocytes and 50 % polymorphonuclear leucocytes. It is reasonable to assume that the peripheral lymphocytes are immuno competent, but it is uncertain which cell is to be considered as the "normal" cell and thus comparable with the macrophages in David's experiments. Investigations to elicit the role of the polymorphonuclear cells and other blood cells in the migration inhibition reaction are in progress. The possible relationship between the proportions of the various cell types and the recorded migration inhibition would be of particular interest.

Little is known about the chemical constitution of the substance elaborated by the sensitized lymphocytes, except that it is non dialyzable and active after heating at 56° C for 30 min. (12).

The possibility that this factor is identical with the transfer factor described by Lawrence (20) is indeed very interesting and might suggest that the phenomenon observed in vitro is closely akin to in vivo reactions.

The migration inhibition method could usefully be compared with the blast cell transformation test which some authors have reckoned to be a means of detecting delayed hypersensitivity in vitro (2, 17, 22). An analysis of the validity of the two methods in this respect must be based upon fulfilment of the following conditions.

1. *Distinction between humoral and cellular hypersensitivity.*

Use of the blast transformation test gave positive results with antigens which typically give rise to humoral hypersensitivity (tetanus, diphtheria toxoid). A blast cell transformation within the same percentage range was elicited with PPD in cell cultures from persons with a positive intracutaneous tuberculin reaction (2, 9).

On the other hand it has not been possible to elicit a migration inhibition with antigens that induce a humoral type of hypersensitivity, as shown very convincingly by David et al. (11).

In view of the lack of correlation between circulating antibodies and the migration indices, the results presented here seem to indicate that the migration inhibition method allows a distinction between the two types of hypersensitivity.

2. *Correlation of the in vitro parameter and the cutaneous reaction.*

Already Pearmain et al. (23) had noted in their original work — and later Hersh and Oppenheim (17) — that there is a poor correlation between the blast transformation and the intracutaneous reaction.

Hirschhorn et al. (18) on the other hand found a fairly good correlation of positive tuberculin tests and in vitro response to PPD. From the published data, however, it is seen that there is no proportionality between the percentage of blast cells and the magnitude of the cutaneous reaction. Passaleva et al. (22) again were able to show a certain correlation between the degree of blast transformation and cutaneous reaction in tuberculous individuals. These authors have divided the tuberculin positive patients into three groups with increasing dermal sensitivity. The mean value of the percentage of blast cells from each group shows a corresponding increase,

but the variation within each group indicates that a considerable overlap must take place Furthermore it can be seen from the observations that some cases with a positive tuberculin test will fall within the range of the normal spontaneous occurrence of blast cells

On the contrary, experiments in animals with the migration test have shown (5 6 11 14, 19) that in all cases of a positive cutaneous test, a corresponding significant inhibition of migration was found This observation has been confirmed by the results in this communication and extended by the demonstration of the proportionality of the in vivo and the in vitro parameter

3 *The time correlation between the in vitro and the in vivo parameter*

The typical delayed cutaneous reaction reaches its maximum after 24—48 hours The transformation of lymphocytes does not reach its maximum until after five days At this time it is possible to detect in and around the cells antibodies of the same type as found in serum (1, 13) The maximal inhibition of migration can be seen after 24 hours (11, 14, 15)

Accordingly the lymphocyte transformation test undoubtedly expresses *some kind* of immunological reaction, but this method cannot distinguish between cellular and humoral hypersensitivity

The reaction apparently has a close relationship with the ability to produce circulating antibodies

In contrast, the specific inhibition of migration of sensitized cells allows a distinction to be made between the two modes of immunological reaction more over is well correlated with the in vivo phenomena This method therefore must be considered the most suitable for detecting delayed hypersensitivity in man

The method presented here is an experimental version with use of brucella bacteria as an antigen but any other antigen with the ability to develop delayed hypersensitivity could probably be substituted The technique may prove valuable in the study of cellular hypersensitivity in auto immune diseases in transplantation immunity and in other fields

Summary

A method for detecting cellular hypersensitivity in vitro in man is presented It is based on specific inhibition of migration of sensitized white blood cells Brucella bacteria were used as antigen The specificity of the method is discussed and compared with that of another in vitro technique the blast transformation test It is concluded that the migration inhibition method gives a specific expression of cellular hypersensitivity and must be considered the most suitable for investigation of cellular hypersensitivity in vitro

Acknowledgements

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Acta Medica Scandinavica Vol 182, fasc 2, 1967

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The Hemodynamic Effect of Changes in Posture in Cardiac Patients

By

EINAR LORENTSEN, GUNNAR BAY, HELGE GRENDAHL and EGIL SIVERTSSEN

Circulatory adaptation on changing from a recumbent to the upright position depends on reflex stimulation of the heart, on reflex vasoregulation, and on adjustment between intra and extra vascular water Despite these circulatory adjustments to the upright position, a significant fall in stroke volume and cardiac output is found in normal subjects (1, 2, 8, 11, 12 14, 15) This decrease is supposed to be due to a gravitational pooling of blood in the lower part of the body with a decrease in central blood volume and in ventricular filling pressure (11) Postural hemodynamic changes seem to be less pronounced in older than in younger subjects (4)

The present investigation deals with the hemodynamic effects of postural changes in patients with different types of cardiac diseases and different degrees of congestive heart failure There is little information about the effects of postural changes in cardiac patients Donald et al (3) found a slight fall in cardiac output in 36 patients with heart diseases, but there were marked individual variations A change from supine to upright position in patients with heart failure often gives no reduction of stroke volume and cardiac output (7, 8, 11, 13)

Submitted for publication January 9 1967

Material and methods

The study was carried out in 22 patients Clinical data are summarized in table I

Cardiac output was determined by a dye dilution technique A polyethylene catheter was inserted percutaneously through an antecubital vein into the superior caval vein or the right atrium A thin teflon-coated electrode with platinum tip was inserted through the catheter and the peripheral end of the electrode was connected to the V-terminal of an electrocardiograph By ECG registration the tip of the catheter could be localized A slow infusion of 5 % glucose in water was given through the catheter to maintain patency and to compensate for the loss of fluid Thereafter a polyethylene tubing was inserted percutaneously through the common femoral artery into the common iliac artery or the distal part of the aorta

Injections of indocyanine green (Cardiogreen manufactured by Hynson Westcott

TABLE II Hemodynamic data in supine (L) and sitting (S) position in 22 patients

Group	No of pats	Position	Cardiac output (l/min Δ %)	
All patients	22	L	4 62	
		S	4 30	— 7
Without or with slight degrees of heart failure	12	L	5 19	
		S	4 59	—12
Advanced degree of heart failure	10	L	4 05	
		S	4 01	— 1
Mainly involvement of left ventricle	9	L	4 18	
		S	3 72	—11
Mainly involvement of right ventricle	4	L	4 36	
		S	4 40	+ 1
Aortic valvular disease	5	L	3 89	
		S	3 28	—16
Tricuspid valvular insufficiency	2	L	4 80	
		S	5 78	+20
Pulmonary emphysema	6	L	4 64	
		S	4 25	— 8

four patients with mainly right heart involvement had a decrease in cardiac output and in stroke volume on changing from the supine to the sitting position The mean systemic arterial pressure increased in both groups Peripheral resistance showed the largest increase in patients with mainly left heart involvement (fig 2)

Anatomical diagnosis

Patients with *aortic valvular diseases* showed the greatest fall in cardiac output on changing from the supine to the sitting position (fig 3) and there was a decrease even when marked heart failure was present Peripheral resistance increased more than in patients with other heart diseases (table II)

In two patients with *tricuspid valvular insufficiency* (cases no 3 and 8) the cardiac output increased by 8 % and 26 % respectively on changing from the supine to the sitting position Stroke volume increased correspondingly The peripheral resistance decreased in one and remained unchanged in the other patient

Patients with *pulmonary emphysema* varied in their response to changes in position Two patients without signs of heart failure (cases no 7 and 18) showed a marked fall in cardiac output In patients with pulmonary emphysema and

Heart rate (beats/min)	Stroke volume (ml/min Δ %)		Mean arterial systemic pressure (mm Hg Δ %)		Peripheral resistance (dynes sec cm⁻⁵ Δ %)	
74	65		91		1 590	
75	59	— 9	101	+11	1 951	+23
76	70		101		1 560	
78	60	—14	112	+11	1 920	+23
71	59		81		1 619	
71	58	— 2	89	+11	1 981	+22
71	63		86		1 683	
72	55	—13	101	+17	2 290	+36
80	64		86		1 587	
82	65	+ 2	101	+17	1 891	+19
78	52		93		1 889	
79	43	—17	109	+17	2 826	+50
70	73		68		1 014	
64	84	+15	70	+ 3	947	— 7
80	62		90		1 526	
81	58	— 6	102	+13	1 831	+20

signs of right ventricular failure only a slight decrease or no change at all was found Mean systemic arterial pressure increased in all these patients except one

Comments

The *postural hemodynamic* changes varied greatly from patient to patient in our study The whole group showed a slight fall in cardiac output This accords with the observation of Donald et al (3) in a similar study

As in other studies (6 7 8 11 13) a decrease in stroke volume and cardiac output was seen in patients without or with only slight heart failure and no significant changes in patients with severe heart failure This lack of change has been attributed to resistance to gravitational pooling of blood in the venous tree in patients with severe heart failure (11)

Patients with aortic valvular disease seem to behave in a special manner In these patients stroke volume and cardiac output decreased also when severe heart failure was present Despite a considerable fall in stroke volume in some of these patients no increase of heart rate was seen Taquini et al (13) found a similar decrease in cardiac output in one patient with aortic valvular

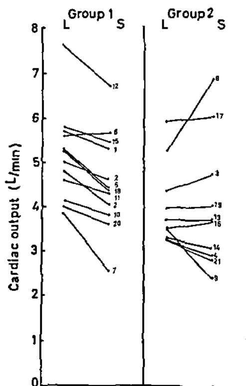


Fig 1 The effect of posture on cardiac output in patients with different degrees of heart failure Group 1 patients without or with a slight degree of heart failure Group 2 patients with a servere degree of heart failure The figure given with each curve refers to the patient s number in table I L lying S sitting

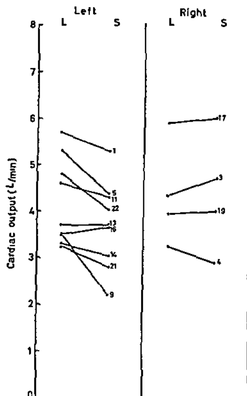


Fig 2 The effect of posture on cardiac output in patients with mainly left heart involvement and in patients with mainly right heart involvement The figure given with each curve refers to the patient s number in table I L lying S sitting

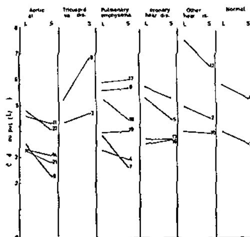


Fig 3 The effect of posture on cardiac output in patients with different types of heart diseases and in patients with pulmonary emphysema The figure given with each curve refers to the patient s number in table I L lying S sitting

insufficiency and severe heart failure whereas in patients with mitral insufficiency an increase in cardiac output was found No explanation for this different behavior is given The tendency to fainting and syncope in patients with aortic valvular disease may be related to this maladjustment to the upright position

The increase in cardiac output in patients with tricuspid valvular insufficiency is of special interest This observation accords with the findings of Taquini et al (13) A marked increase in cardiac output has also been found after venesection in patients with severe heart failure due to mitral valvular disease and functional insufficiency of the tricuspid valves (7) McMichael and Schillingford (9) have postulated that this increase is due to a reduced regurgitation of blood as a consequence of reduced venous return and reduced dilatation of the right ventricle in the upright position The descending limb of the Starling curve may be due to such regurgitation through the atrioventricular valves (10)

In patients with pulmonary emphysema the response to changes in posture varied This is probably due to different degrees of pulmonary hypertension and of right ventricular failure Similar observations have been made in studies on the effect of positive pressure breathing (16)

Summary

The hemodynamic effects of changes from a supine to a sitting position have been demonstrated in 17 patients with heart diseases three patients with pulmonary emphysema without heart failure and two normal subjects

In patients without or with only slight degree of heart failure the stroke volume and the cardiac output decreased in all except one In patients with severe heart failure marked individual variations were found In five patients with aortic valvular disease the stroke volume and the cardiac output decreased significantly even when severe heart failure was present In two patients with tricuspid valvular insufficiency the stroke volume and cardiac output increased on changing from the supine to the sitting position Possible explanations of these observations are discussed

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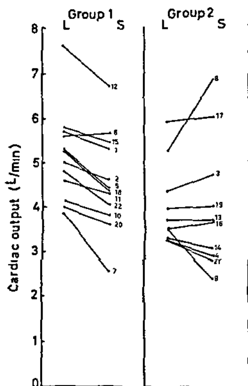


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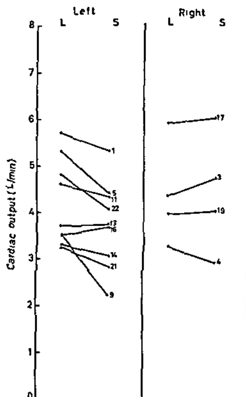


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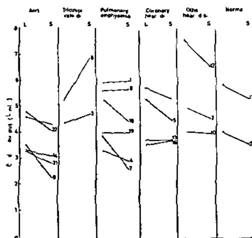


Fig 3 The effect of posture on cardiac output in patients with different types of heart diseases and in patients with pulmonary emphysema The figure given with each curve refers to the patient s number in table I L lying S sitting

Acta Medica Scandinavica Vol 182, fasc 2, 1967

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Studies on Hemoglobin Values in Norway

VII Hemoglobin, hematocrit and MCHC values among boys and girls aged 7—20 years in elementary and grammar schools

By

HAAKON NATVIG ODD D VELLAR and JAKOB ANDERSEN

In an earlier investigation (10) it was demonstrated that in school children aged 10—13 years who had received a large enough dose of supplementary iron for a long enough time, nearly all sub normal hemoglobin (Hb) values disappeared, and the mean Hb concentration increased On this basis a value of 13 2 g% ± 1 7 was considered normal for Norwegian school children aged 10—13 years

Other studies (6 8, 11) have shown that mild anemia is not rare among men and also indicate that MCHC values might be a more sensitive indicator of mild iron deficiency anemia than Hb concentration as an isolated parameter

In order to make the investigation of Hb values in Norway more complete, the present study deals with Hb hemato crit (Hct) and MCHC values in school children aged 7—20 years in separated age groups

Submitted for publication January 23 1967

Material and methods

The present material was obtained at one elementary school and at two grammar schools in Oslo The age and sex distributions are shown in table I

The blood samples were taken from the finger tip by pricking with lancets and the Hb and Hct determinations were performed by the method described in an earlier study (6) All the blood samples were taken and the readings performed by the same well trained nurse

Results

A Descriptive part

Hb concentrations

Table II shows that there is no significant sex difference in Hb values prior to 15 years of age From the age of 7 to 13, the values increase moderately in both sexes From then on there is a more rapid increase in boys to the age of 20 in girls a corresponding increase

TABLE I Age and sex distributions of the pupils examined

Pupils elementary school				Pupils grammar schools			
Age	Boys	Girls	Total	Age	Boys	Girls	Total
7	25	29	54	14	2	7	9
8	28	22	50	15	80	83	163
9	27	23	50	16	90	83	173
10	84	80	164	17	67	91	158
11	19	25	44	18	121	108	229
12	33	37	70	19	107	77	184
13	34	37	71	20	60	32	92
Total	250	253	503	Total	527	481	1 008

TABLE II Mean hemoglobin concentration and S D in the different age groups for boys and girls in the elementary and grammar schools

Pupils elementary school					Pupils grammar schools				
	Boys mean		Girls mean			Boys mean		Girls mean	
Age	g %	S D	g %	S D	Age	g %	S D	g %	S D
7	12 54	0 83	12 47	0 90	14	14 10	—	14 14	—
8	13 00	0 73	12 93	0 78	15	14 70	1 06	14 11	1 15
9	12 51	0 60	12 63	0 73	16	14 96	1 07	13 87	1 08
10	13 13	0 82	13 24	0 69	17	15 29	1 10	13 86	0 94
11	13 58	0 84	13 46	0 81	18	15 45	0 82	13 93	1 22
12	13 61	0 90	13 61	0 81	19	15 48	0 90	14 04	0 84
13	13 36	0 94	13 31	0 84	20	15 70	1 08	13 98	0 94

is seen between the age of 13 and 14 but not later The Hb values for the girls aged 14 are continued into adult life without further increase (fig 1)

The frequency distribution of the Hb values in different age groups is presented in fig 2 In boys the distribution curve is shifted towards higher values with increasing age A corresponding shift is demonstrated in the girls until the age of 14—16 but not later and there is a tendency to even lower values in the 19—20 years age group

The Hct values (table III, fig 3) accord rather well with the Hb values Here also there is no difference between the sexes before the age of 15 A slight increase is noted from the age of 7 up to

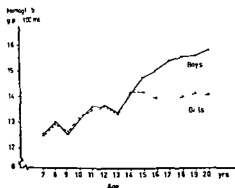


Fig 1 Mean hemoglobin concentrations by age in boys and girls

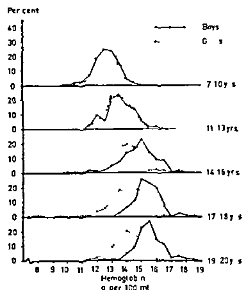


Fig 2 The relative distribution of hemoglobin concentrations in age groups in boys and girls

TABLE III Mean hematocrit values and S D in the different age groups for boys and girls in the elementary and grammar schools

Pupils elementary school					Pupils grammar schools				
	Boys mean		Girls mean			Boys mean		Girls mean	
Age	%	S D	%	S D	Age	%	S D	%	S D
7	38 9	1 97	38 6	2 83	14	41 0	—	41 6	—
8	39 4	2 85	39 8	1 97	15	43 1	3 33	41 0	2 95
9	37 3	2 43	39 9	3 29	16	43 7	3 26	41 1	3 17
10	39 3	3 23	39 7	2 74	17	45 2	3 75	41 1	3 10
11	40 8	2 45	40 2	2 58	18	45 2	3 37	41 7	3 24
12	40 7	2 44	41 0	3 18	19	45 4	3 04	41 7	2 95
13	41 2	2 36	40 4	2 77	20	45 6	3 16	41 3	2 58

14 from then on there is a marked increase in boys whereas the values in girls have remained at the 14 year level

The relative distribution of the Hct values is presented in fig 4 The curves illustrate a shift towards higher values in both sexes from the age of 7—10 to 11—13 After this age there is a further shift towards higher values in boys with a maximum reached at the age of 17

TABLE I Age and sex distributions of the pupils examined

Pupils elementary school				Pupils grammar schools			
Age	Boys	Girls	Total	Age	Boys	Girls	Total
7	25	29	54	14	2	7	9
8	28	22	50	15	80	83	163
9	27	23	50	16	90	83	173
10	84	80	164	17	67	91	158
11	19	25	44	18	121	108	229
12	33	37	70	19	107	77	184
13	34	37	71	20	60	32	92
Total	250	253	503	Total	527	481	1 008

TABLE II Mean hemoglobin concentration and S D in the different age groups for boys and girls in the elementary and grammar schools

Pupils elementary school					Pupils grammar schools				
	Boys mean		Girls mean			Boys mean		Girls mean	
Age	g %	S D	g %	S D	Age	g %	S D	g %	S D
7	12 54	0 83	12 47	0 90	14	14 10	—	14 14	—
8	13 00	0 73	12 93	0 78	15	14 70	1 06	14 11	1 15
9	12 51	0 60	12 63	0 73	16	14 96	1 07	13 87	1 08
10	13 13	0 82	13 24	0 69	17	15 29	1 10	13 86	0 94
11	13 58	0 84	13 46	0 81	18	15 45	0 82	13 93	1 22
12	13 61	0 90	13 61	0 81	19	15 48	0 90	14 04	0 84
13	13 36	0 94	13 31	0 84	20	15 70	1 08	13 98	0 94

is seen between the age of 13 and 14 but not later The Hb values for the girls aged 14 are continued into adult life without further increase (fig 1)

The frequency distribution of the Hb values in different age groups is presented in fig 2 In boys the distribution curve is shifted towards higher values with increasing age A corresponding shift is demonstrated in the girls until the age of 14—16, but not later, and there is a tendency to even lower values in the 19—20 years age group

The Hct values (table III, fig 3) accord rather well with the Hb values Here also there is no difference between the sexes before the age of 15 A slight increase is noted from the age of 7 up to

TABLE IV Mean MCHC values and S D in the different age groups for boys and girls in the elementary and grammar schools

Pupils elementary school					Pupils grammar schools				
	Boys mean		Girls mean			Boys mean		Girls mean	
Age	%	S D	%	S D	Age	%	S D	%	S D
7	32 3	1 43	32 4	1 98	14	34 4	—	34 1	—
8	33 1	2 04	32 5	1 99	15	34 1	2 27	34 8	2 76
9	33 6	1 71	31 8	2 01	16	34 3	2 08	33 8	2 17
10	33 5	2 13	33 4	1 92	17	33 9	1 93	33 8	2 04
11	33 3	2 19	33 5	1 22	18	34 3	1 98	33 4	2 00
12	33 4	1 60	33 2	1 93	19	34 2	1 76	33 7	1 91
13	32 5	2 38	33 0	1 66	20	34 5	1 97	33 8	1 45

TABLE V Mean hemoglobin hematocrit and MCHC values before and after supplementary iron in boys and girls aged 14—20 years

		Consumed no of iron tablets (mean)	Hb mean (g %)			Hct mean (%)			MCHC mean (%)		
			Before iron	After iron		Before iron	After iron		Before iron	After iron	
				1 mo	2—3 mos		1 mo	2—3 mos		1 mo	2—3 mos
Therapy group	12 boys	224	13 5	14 8	15 4	46 2	43 9	44 5	29 3	33 8	34 7
	17 girls	162	12 1	13 5	14 1	41 8	40 8	41 8	28 8	33 1	33 7
Control group	43 boys	146	15 2	15 4	15 5	44 2	44 3	45 0	34 5	34 7	34 6
	45 girls	144	13 8	13 9	14 2	40 5	40 6	41 0	34 1	34 2	34 6

18 There is no corresponding increase in girls after the age of puberty

The MCHC values (table IV fig 5) exhibit an irregular pattern, but with a tendency to a slight increase from the age of 7 up to 14 After this age the level is fairly constant but with somewhat lower values in girls aged 18 to 20 compared with boys of the same age

The relative distributions of the MCHC values (fig 6) remain on the same level in the different age groups and are almost equal in the two sexes

B Experimental part

1 The therapy trial

All pupils aged 14—20 years with MCHC values below 30 5 % received iron supplements and they were re examined one two and three months later The supplementary iron was given

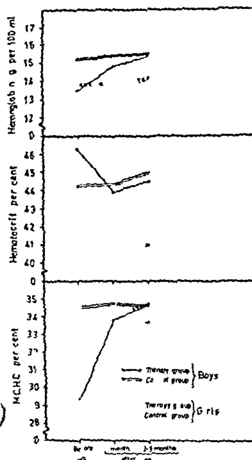


Fig The response to iron supplements in the therapy groups and the control groups in boys and girls

in the form of ferrofumarate tablets (Neo-Fer Nyco supplied by Nyegaard & Co Oslo One tablet is equivalent to approximately 60 mg of bivalent iron Each pupil received the tablets packed in boxes of 100 tablets and was instructed to take one tablet three times a day When the person was seen after one month's interval the actual consumption of tablets in the period was recorded and another box of tablets was handed out

The number of subjects in the therapy group were 12 boys and 17 girls The mean MCHC before iron supplementation was 29 3 % with a minimum value of 26 9 %

Table V demonstrates clearly the increase in both Hb and MCHC values as a response to iron therapy in boys as well as in girls The Hct values have a tendency to decrease in boys whereas in girls they are kept at a fairly constant level

2 The control trial

In order to evaluate whether or not the Hb Hct and MCHC values found in adolescent boys and girls aged 14—20 years were the physiological optimal values a systematic sample of one in ten of the original material was given supplementary iron according to the same scheme as adopted in the therapy group — regardless of the blood values obtained at the first examination The control group consisted of this 10 % sample and included 43 boys and 45 girls fairly evenly distributed among the different age groups Eighteen boys and 16 girls consumed only one month's ration of iron tablets and were for this reason re examined only once whereas 25 boys and 29 girls continued the iron supplement for 2—3 months and were re examined after each interval

Table X shows a small but consistent increase of the Hb concentration in both boys and girls as a response to the iron supplements An analogous increment in Hct and MCHC values was also observed Fig 7 illustrates that the therapy group as well as the control group after a 2 3 month period of supplementary iron reached a mean Hb concentration of 15.5 g% in adolescent boys and of

14 2 g% in adolescent girls The final Hct level is 44.5 % in boys and 41 5 % in girls The endpoint of the MCHC values is in both sexes 34 5 %

Discussion

The results of the present study have confirmed previous findings (9, 10 13) of no sex difference in blood parameters in the age group 7 to 14 years, whereas a substantial difference between boys and girls is found after the age of 14 This tallies with other sex differences which start at puberty

There is a moderate increase in both Hb and Hct values from the age 7 up to 14 In boys the increase continues to the age of 20 whereas girls remain at the 14 year level from then on There seems to be no obvious sex difference in the MCHC values There is a tendency to a slight increase from the age 7 up to 14 in both sexes but thereafter the level is fairly constant

On the basis of a previous study of school children aged 10—13 years who had received iron supplements (10) and of the results of the present investigation we find it justified to propose the data shown in table VI as normal values for Hb Hct and MCHC in Norwegian boys and girls aged 7—20 years As average values are taken the mean values exhibited by the boys and girls after they had received an adequate supplement of iron As normal ranges are taken these average values plus or minus a value comparable to twice the standard deviation found in the descriptive part of this study Values below the lower limit of this range usually indicate iron deficiency anemia with a need for iron supplements

Table VII shows the frequency of iron deficiency anemia in boys and girls in different age groups according to these criteria Whereas anemia seems to be of the same frequency in boys and girls aged 7 to 13 it is found more often in girls aged 14 to 20 than in boys of the same age Boys and girls aged 14 to 16 years appear to be particularly prone to iron deficiency anemia Cases with severe anemia (in one boy 10 4 g% Hb, in girls 8 0 9 6 10 0, 10 6 11 1 and 11 5 g% Hb) were detected in grammar school pupils who worked hard and who thought that their physical and mental fatigue were natural for their age When they reached normal Hb values as a response to iron supplementation for the first time they really felt how it was to be healthy and fit

Subnormal MCHC values (table VIII) are generally found more frequently than subnormal Hb values, in both sexes

Our proposals for normal values and consequently the criteria for what should be considered as anemia are largely in agreement with those reported earlier both by Scandinavian (1 3 4, 12 14 15) and by other workers (2, 5) We reckon that normal values i e the physiological and optimal values for Hb, Hct and MCHC, must be based on data from apparently healthy persons who have received an adequate dose of supplementary iron for a long enough period of time to rule out iron deficiency In accord with previous results in school children aged 10—13 years (11), the present study demonstrates that iron

TABLE VI Proposed normal values of hemoglobin, hematocrit and MCHC for Norwegian boys and girls aged 7—20 years

Age	Boys						Girls					
	Hb mean		Hct mean		MCHC mean		Hb mean		Hct mean		MCHC mean	
	g %	range	%	range	%	range	g %	range	%	range	%	range
7—9	12.7	±1.6	39	±5.0	33	±4	12.7	±1.6	39	±5.0	33	±4
10—13	13.2	±1.6	40	±5.0	33	±4	13.2	±1.6	40	±5.0	33	±4
14—16	15.0	±2.0	43	±6.0	34	±4	14.2	±2.0	41	±6.0	34	±4
17—20	15.5	±2.0	45	±6.0	34	±4	14.2	±2.0	42	±6.0	34	±4

TABLE VII The frequency of iron-deficiency anemia in boys and girls aged 7—20 years

Age	Boys			Girls		
	Hb conc. below (g %)	No	%	Hb conc below (g %)	No	%
7—10	11.0	2	0.6	11.0	2	0.6
11—13	11.5	4	2.1	11.5	4	2.1
14—16	13.0	5	2.9	12.0	6	3.4
17—20	13.5	2	0.7	12.0	6	1.7

TABLE VIII The frequency of subnormal MCHC values in boys and girls aged 7—20 years

Age	MCHC below	Boys		Girls	
	%	No	%	No	%
7—10	29	3	1.7	5	3.2
11—13	29	4	4.7	2	2.0
14—16	30	6	3.5	6	3.5
17—20	30	8	2.3	11	3.6

supplementation tends to increase the mean Hb values in boys and girls aged 14—20 years, and that almost all low values disappear

In agreement with previous findings in young men (6, 11), the present investigation indicates that the MCHC value is a more sensitive parameter in

Summary

In this study of [illegible] elementary school pupils aged 7–13 years and [illegible] grammar school pupils aged 13–20 years in Oslo no sex difference in Hb and MCHC values was found before the age of 13. In both sexes there is a gradual increase from 7 to 13 years, with further increase in boys up to the age of 20, whereas no corresponding increase in the values for girls could be found after puberty. The MCHC values, however, are roughly equal in both sexes.

In order to reveal whether or not the observed low Hb and MCHC values were due to iron deficiency, all persons with MCHC below 30.5 % received 60 mg of bivalent iron in the form of ferro-fumarate tablets three times a day for 2–3 months. In addition a 10 per cent sample drawn systematically from the original material followed the same scheme for iron supplementation, regardless of blood values found at the initial examination. In both groups the Hb as well as the MCHC values reached th

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Studies on Hemoglobin Values in Norway

VIII Hemoglobin, hematocrit and MCHC values in adult men and women

By

Haakon Natvig and Odd D Vellar

In an attempt to obtain up-to-date information about the distribution of hemoglobin (Hb), hematocrit (Hct) and MCHC values in the Norwegian population a number of surveys have been conducted in different population groups (18 19, 20 21 22) We report in this paper the results of our survey in adult men and women with the purpose of proposing normal values and reporting the prevalence of iron deficiency anemia

Material and methods

The persons examined were employed in an electrotechnical company in Oslo where one of the authors (H N) is industrial medical officer Blood samples for the determination of Hb and Hct were taken from all persons who were seen by the medical officer in connection with pre employment or periodic medical examinations A record was completed on the basis of the medical officer s knowledge of the general health and sick leave records of each individual In addition a personal interview was conducted in order to assess anemia in the past and to record conditions associated with blood loss such as accidents and operations gastrointestinal hemorrhage blood donations and for women large menstrual bleedings etc The intake of supplementary iron was also recorded The investigation was carried out from Jan 1964 to July 1966

The age and sex distributions of the total material are shown in table I In the age group 60 years and over three men and one woman were between 70 and 73 All the others in the group were between 60 and 69 years

In order to study the blood values in apparently healthy men and women 111 men and 54 women were excluded as persons with clinical diseases with blood loss and with definitely abnormal Hb and MCHC values The age and sex distribution of the excluded individuals as well as the composition of the normal material are presented in table I The detailed reasons for the exclusion are seen in table VII and will be commented on later

The blood samples were taken by venepuncture from the cubital vein and the Hb and Hct determinations were performed with the method described in an earlier study (18) All the blood samples were taken and

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TABLE I The age and sex distributions of the total material the excluded individuals and the normal material

Age	Men			Women		
	Total	Excluded	Normal	Total	Excluded	Normal
15—19	122	3	119	39	4	35
20—29	323	29	294	88	17	71
30—39	176	15	161	32	8	24
40—49	210	18	192	76	12	64
50—59	172	25	147	59	10	49
>60	114	21	93	28	3	25
Total	1 117	111	1 006	322	54	268

TABLE II Mean hemoglobin hematocrit and MCHC values with S D in the different age groups for men and women in the total material

Age	Men						Women					
	Hb mean		Hct mean		MCHC mean		Hb mean		Hct mean		MCHC mean	
	g %	S D	%	S D	%	S D	g %	S D	%	S D	%	S D
15—19	15 51	0 81	46 02	3 20	33 61	1 64	14 37	0 82	42 81	2 66	33 67	1 71
20—29	15 66	0 91	46 34	3 00	33 82	1 69	14 47	0 88	42 50	2 84	33 31	2 29
30—39	15 78	0 93	46 25	2 96	33 55	1 66	14 00	1 22	42 12	3 57	33 19	2 24
40—49	15 56	0 92	46 42	3 34	33 55	1 70	14 14	1 09	42 24	3 16	33 20	2 27
50—59	15 53	1 10	46 94	3 66	32 86	1 89	14 04	0 84	42 43	2 83	33 19	2 19
>60	15 40	1 10	46 75	3 58	33 25	1 73	14 21	0 90	43 21	2 45	32 93	1 38
Total	15 62	0 96	46 47	3 26	33 50	1 74	14 15	1 02	42 62	2 94	33 22	2 11

the readings performed by the same well trained industrial nurse

Results

1 The total material

The mean values of Hb, Hct and MCHC with S D among the total of men and women examined in the different age groups are presented in table II In men the highest Hb levels were seen between 20 and 39 years with a tendency to lower and more widely deviating values with increasing age The women reached their highest Hb concentration in the age group 20—29 years with relatively low values between 30 and 59 years

The mean Hct values show little variation from age group to age group, there

TABLE III a The distribution of hemoglobin concentration with mean and S D among men and women in different age groups in the normal material

Hb (g %)	Men (yrs)							Women (yrs)						
	15—19	20—29	30—39	40—49	50—59	>60	Total	15—19	20—29	30—39	40—49	50—59	>60	Total
12 0—12 9	1						1	2	4	1	1	3	1	12
13 0—13 9	2	1		5	3	4	15	7	18	8	19	22	7	81
14 0—14 9	23	43	22	32	29	23	172	20	37	10	35	19	13	134
15 0—15 9	64	146	82	103	68	39	502	5	11	4	7	5	3	35
16 0—16 9	26	84	40	41	37	19	247	1	1	1	2		1	6
17 0—17 9	3	17	13	9	9	7	58							
18 0—18 9		3	4	2	1	1	11							
No in group	119	294	161	192	147	93	1 006	35	71	24	64	49	25	268
Mean	15 5	15 8	15 9	15 6	15 7	15 6	15 7	14 4	14 3	14 3	14 3	14 0	14 3	14 3
S D	0 81	0 83	0 90	0 87	0 90	1 01	0 88	0 84	0 82	0 92	0 76	0 77	0 85	0 81

TABLE III b The distribution of hematocrit values with mean and S D among men and women in different age groups in the normal material

Hct (%)	Men (yrs)							Women (yrs)						
	15—19	20—29	30—39	40—49	50—59	>60	Total	15—19	20—29	30—39	40—49	50—59	>60	Total
33—36										1	1			2
37—40	6	6		3	5	2	22	7	15	6	17	12	2	59
41—44	28	57	33	52	24	22	216	19	42	13	33	30	17	154
45—48	60	166	90	89	76	45	526	9	13	3	13	5	6	49
49—52	25	61	35	45	35	21	222		1	1		2		4
53—56		4	2	3	7	3	19							
57—60			1				1							
No in group	119	294	161	192	147	93	1 006	35	71	24	64	49	25	268
Mean	46 0	46 5	46 7	46 4	46 9	46 5	46 5	42 7	42 5	42 0	42 1	42 3	43 1	42 4
S D	3 20	2 94	2 90	3 18	3 40	3 31	3 11	2 73	2 70	3 40	2 91	2 88	2 22	2 81

are, however slightly higher values in men over the age of 50 and in women under 30

The mean MCHC values in men differ only moderately from age group to age group but with a tendency towards relatively lower values in men over 50 years Women aged 15—19 years have MCHC values comparable to those of men of the same age In all other age

TABLE V a Mean hemoglobin hematocrit and MCHC values among men in the control group before and after one two and three months of iron supplementation

	62 men 15—39 yrs 185 tabl on average			40 men 40—69 yrs 215 tabl on average			102 men 15—69 yrs 197 tabl on average		
Time of examination	Hb mean (g %)	Hct mean (%)	MCHC mean (%)	Hb mean (g %)	Hct mean (%)	MCHC mean (%)	Hb mean (g %)	Hct mean (%)	MCHC mean (%)
Before iron	15 7	46 7	33 6	15 4	46 8	32 8	15 6	46 8	33 3
After iron									
1 mo	15 7	46 9	33 5	15 3	46 0	33 2	15 6	46 6	33 3
2 mos	15 6	46 2	33 9	15 5	46 1	33 5	15 6	46 2	33 7
3 mos	15 7	47 1	33 4	15 4	45 9	33 5	15 6	46 6	33 4

TABLE V b Mean hemoglobin hematocrit and MCHC values among women in the control group before and after one two and three months of iron supplementation

	21 women 15 39 yrs 197 tabl on average			19 women 40 69 yrs 174 tabl on average			40 women 15 69 yrs 186 tabl on average		
Time of examination	Hb mean (g %)	Hct mean (%)	MCHC mean (%)	Hb mean (g %)	Hct mean (%)	MCHC mean (%)	Hb mean (g %)	Hct mean (%)	MCHC mean (%)
Before iron	14 0	42 7	32 9	13 8	42 2	32 6	13 9	42 4	32 7
After iron									
1 mo	14 1	43 0	32 9	14 1	42 6	33 0	14 1	42 8	33 0
2 mos	14 3	43 5	33 3	14 1	42 3	33 6	14 2	42 9	33 4
3 mos	14 2	42 3	33 5	14 2	42 4	33 5	14 2	42 4	33 5

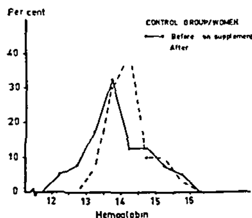


Fig 1 The relative distributions of hemoglobin concentration among the women in the control group before and after three months of iron supplementation

centration in women before and after 2—3 months of supplementary iron is presented in fig 1 The curves illustrate a shift towards higher values as well as a disappearance of values below 13 0 g% A comparable shift is seen in the MCHC values

4 Supplementary iron given to persons with MCHC < 30.5 % — the therapy trial

In order to evaluate the influence of iron supplement on the blood levels of individuals with subnormal MCHC val

ues all persons with MCHC below 30 5 % were given iron tablets after the same scheme as adopted in the control group Altogether, 25 men and 23 women completed this trial The mean Hb Hct and MCHC values before and after iron supplementation are shown in table VI In both sexes a substantial increase in Hb concentration as well as in MCHC is achieved The relative distributions of Hb concentrations before iron medication as shown in figs 2 and 3, become normalized and the long tail of lower values is eliminated It is remarkable that Hb, Hct and MCHC values in men, as well as in women, reach identical end points in both the control group and the therapy group as demonstrated in fig 4 The therapy trial indicates clearly that low MCHC values are corrected with iron supplementation which also gives higher Hb levels

Among the 25 men with MCHC below 30 5 % only three had a Hb concentration below 14 g% and 11 had more than 15 g% *Iron deficiency in these 11 men would not have been recognized unless MCHC had been calculated* Only eight women among the 23 in the therapy group had Hb values below 12 5 g% i e only one third of the female cases with iron deficiency would have been diagnosed without the use of the MCHC parameter

5 The excluded individuals

A total of 111 men and 54 women were excluded from the normal material (table I) Of these, 25 men and 23 women had MCHC below 30 5 % and were included in the therapy group (vide supra) In table VII, the other excluded individuals are presented separated into sub groups according to the different reasons for the exclusion As the grouping shows, these excluded men and women have lower Hb Hct and MCHC values than found in the normal material There are in addition some differences among the sub groups but the numbers in most instances are too *small to give conclusive information*

TABLE VI Mean hemoglobin hematocrit and MCHC values among men and women in the therapy group before and after one two and three months of iron supplementation

	25 men 225 tablets on average			*23 women 194 tablets on average*		
Time of examination	Hb mean (g %)	Hct mean (%)	MCHC mean (%)	Hb mean (g %)	Hct mean (%)	MCHC mean (%)
Before iron	14 54	48 7	29 6	12 88	43 8	29 0
After iron						
1 mo	15 42	45 8	33 8	13 80	42 3	32 7
2 mos	15 37	46 1	33 5	13 78	42 6	32 2
3 mos	15 67	46 0	34 0	14 25	42 2	33 6

Some of those excluded have received iron supplements with a good response in all but three persons

6 Proposed normal values

The mean values of Hb Hct and MCHC found in the normal material are nearly identical with those of the control group and the therapy group after iron supplementation In our opinion it is therefore justified to regard these values as optimal and physiological i e as *normal values* On the basis of the very minute differences in hematological indices between the different age groups in the normal material it seems reasonable to recommend only one set of normal values for all adult men under the age of 0 and another set for all adult women under the same age

Accordingly proposed normal mean values of Hb Hct and MCHC for adult men and women under 70 years are given in table VIII These means plus or minus a value comparable to twice the standard deviation found in the normal material are taken as normal ranges

Anemia is considered to exist when *the Hb concentration is less than 14 g%* in adult men and less than 12 5 g% in adult women under the age of 70 Such low levels indicate the need for iron treatment Pregnant women however may not be designated as anemic according to this rule

Hct values under 40 % in men and under 36 % in women as well as MCHC values below 30 5 % in both sexes must be considered pathological

7 The frequency of iron deficiency anemia

The frequency of iron deficiency anemia in the total material according to our criteria is shown in table IX In men Hb concentrations below 14 g% are most frequently found after the age of 40 and under the age of 20 In women however Hb concentrations below 12 5 g% are prevalent in all age groups with a peak between 30 and 39 years

In men MCHC values below 30 5 % are not recorded more frequently than subnormal Hb values the average incidence being 3 % for both indices In women however subnormal MCHC values are registered with a substantially higher frequency than subnormal Hb values Thus MCHC used as an isolated hematological index is apparently a better measure of iron deficiency in women than is Hb concentration

Discussion

It would have been most valuable if this survey could have been undertaken in a random sample representative of the general population In such a group however the completion of the control trial and the therapy trial would have been impracticable Furthermore the analysed material has also other assets such as the existence of detailed medical records of all participants From a theoretical point of view the results of this investigation might be relevant only to the status of the employees of one particular industrial company in Oslo There is no reason to suspect however that these individuals differ substantially from the majority of the general popula

tion in conditions of living financial status dietary habits health conditions etc

This study has confirmed previous findings (2 12 13 17, 20 29) that the Hb level is maximal in men aged 20—39 years with lower values for each 10-year age group thereafter The difference in the mean values of the Hb concentration between the age groups 20—29 and 60—69 years recorded as 3 9 % in an earlier study (20) is 1 7 % in our present total material and only 1 2 % in the normal material Thus it may be inferred that the lower Hb values of older men to a great extent are due to pathological disorders and that the physiological fall of Hb with increasing age is not as important as supposed The studies of Gillum and Morgan (10) and of Orchard (26) support this view

The Hb values of the total material are also noticeably lower in women than those of the normal material being most marked in the 30—39 year age group

Our proposed means for normal values are in agreement with values found in a few materials comprised of specially selected normal persons (1 4 14 19) It must be said however that our normal values are on a higher level and that our criteria for what should be designated as anemia are more rigorous when compared with our own previous findings (20 24) and the majority of other recent studies (2 3 5 6 7 8 11 12 13 15 16 17 28) In our opinion this discrepancy may be caused by the fact that the materials of these studies have not been thoroughly screened in order to detect and exclude pathological conditions moreover a proportion of the apparently healthy individuals might have suffered from unknown iron deficiency

This study has confirmed previous findings (17 19 24) which indicate that the normal range for MCHC given in hematological text books (30) is too narrow We have found that MCHC values in the region of 30.5—32 0 % are within our normal range Moreover MCHC values above 36 5 % have been registered in 4 1 % of the men and 5 6 % of the women in the normal material It has not been possible to detect any pathological conditions in these individuals

Persons with MCHC values below 30 5 % reached after iron supplements the Hb and MCHC levels of the normal material Also individuals excluded from the normal material demonstrated after supplementary iron increased Hb and MCHC values well inside the normal range Only two men and one woman did not respond to iron treatment their iron deficiency not being an etiological factor The most likely explanation of these findings is that nearly all persons with sub-normal Hb values suffer from iron deficiency

Our previous studies have disclosed that iron deficiency is rather prevalent among school children 23) adolescent grammar school pupils (25) and young men (18 24) Women in the reproductive age and older men also show a high frequency of iron-deficiency anemia as demonstrated in the present study Thus it may be concluded that large sections of the Norwegian population have an inadequate dietary intake of iron This is in agreement with the results of recent

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Ileostomy in Chronic Renal Failure

By

B Lindqvist and B O Oden

Salisbury (6) reported the results of ileostomy in four patients with chronic renal disease and uraemia In all the cases the concentrations of urea, nitrogen uric acid and creatinine in the blood were reduced the blood pressure became normal papillary oedema disappeared and electrolyte balance was improved The amounts of creatinine excreted through the ileostomy were larger than those excreted via the urine in normal individuals The patients could be discharged from hospital The magnitude of the losses of water electrolytes, and nitrogenous products was not stated in the brief report nor were the survival times of the patients

A normal person excretes in the urine per 24 hours 1000—2000 ml of water 80—240 mEq of sodium 50—90 mEq of potassium, 1—10 mEq of calcium 100—150 mEq of chlorides 0 5—0 8 g of phosphorus, 8—10 g of urea nitrogen and 0 8—1 6 g of creatinine and in the faeces 40—200 ml of water 1—10 mEq of sodium 3—15 mEq of potassium about 5 mEq of calcium 2—16 mEq of chlorides, about 0 5 g of phosphorus and 0 25 g of nitrogenous products (3 8 The excretion varies with the diet body weight and metabolism and can be much above or below these figures

An ileostomy patient operated on for ulcerative colitis loses through the stoma per 24 hours 500—1500 ml of water 60—180 mEq of sodium 6—18 mEq of potassium, 50—150 mEq of chlorides and 0 5—1 8 g of nitrogenous products The higher figures refer to the time immediately after the operation gradually the volumes of faeces and thus the electrolytes decrease to the lower values (1 2 3 5 8)

In chronic renal failure the excretion from an ileostomy can be expected to replace in part the function of the kidneys at least as far as water sodium and chlorides are concerned The excretion of potassium and nitrogenous products will presumably be insufficient the question whether and how much the excretion of these substances increases from the stoma at high concentrations in the blood has not hitherto been clarified

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TABLE I Excretion of water electrolytes and nitrogenous products via kidneys and intestine in normal individuals and in ileostomy patients with ulcerative colitis and renal failure respectively

		Normal subjects		Ileostomy patients with	
		Urine	Faeces	Ulcerative colitis Ileostomy fluid	Renal failure Ileostomy fluid
Water	(ml)	1 000—2 000	40—200	500—1 500	800—2 000
Na	(mEq)	80— 240	1— 10	60— 180	120— 290
K	(mEq)	30— 90	3— 15	6— 18	16— 40
Ca	(mEq)	1— 10	ca 5		2— 10
Cl	(mEq)	100— 150	2— 16	50— 150	70— 170
P	(g	0 5— 0 8	ca 0 5		0 2— 0 4
Urea N	(g	8— 10	ca 0 25	0 5— 1 8	0 6— 1 4
Creatinine	(g)	0 8— 1 6			0 1— 0 3

We have performed ileostomies in two patients suffering from chronic renal failure the objective being to try to relax the dietary regimen and reduce the number of haemodialyses

Case reports

Case 1

A 44 year old tractor driver with recurrent duodenal ulcer fell ill in June 1965 with a cough fatigue and dyspnoea One month after the onset of illness he was admitted to another hospital where hypertension enlargement of the heart and uraemia creatinine 23 mg 100 ml of serum were noted

He underwent peritoneal dialysis twice and was then transferred to the medical clinic at Umeå hospital for haemodialysis On admission he was cyanotic and had generalized oedema B P 230 135 mm Hg papillary oedema and haemorrhages in the eye fundi size of the heart 1360 ml sqm body surface Selective renal angiography showed several large cysts in the left kidney the right kidney was small 10 cm in length

In view of the acute onset and the patient's previous physical vigour treatment by intermittent haemodialyses was considered indicated He was haemodialysed twice a week for two months His urinary output was less than 100 ml/24 hours His B P fell to an average of 160/110 mm Hg and slight regression of the fundus changes was noted The heart volume decreased to 1130 ml/sqm body surface His general condition deteriorated, however and he lost weight by 14 kg He was much distressed by the low salt and protein diet and by the necessary fluid restriction An attempt to relieve the uraemia by a protein-free diet supplemented with 10 g of amino acid by mouth daily had to be abandoned as he could not stand this diet more than three weeks We decided to perform an ileostomy in order to increase the excretion of water, electrolytes and nitrogenous products

At the operation the ileum was divided one m from the ileocecal valve The distal end was closed the oral end was drawn through the abdominal wall and an ileostomy was constructed a m Brooke The immediate postoperative course was free from complications Five weeks after the operation however prolapse of the ileostomy necessitated reoperation with revision of the ileostomy The patient also tolerated this procedure well

The excretion from the stoma started on the second post operative day The volumes

of faeces were largest in the second week after operation averaging 2040 ml/24 hours Thereafter they decreased gradually averaging 1040 ml in the 8th week. The concentrations of electrolytes and nitrogenous products varied markedly from day to day but on the average in significantly from week to week The mean concentration of sodium was 131 (80—120) of potassium 23 (13—48) of calcium 3 8 (0 7—9 2) of chlorides 64 (33—96) mEq/l of phosphorus 22 (13—48), of urea nitrogen 82 (29—164) and of creatinine 11 (5—20) mg/100 ml of ileostomy fluid

After establishment of the ileostomy the patient was allowed to take salt and fluid freely Sodium-carbonate 60 (30—240) mEq daily was given to replace the losses of sodium Haemodialysis treatment was necessary because of insufficient excretion of pota sium urea nitrogen and especially creatinine In the first post-operative month the number of dialyses could be limited to one per week But as the excretion from the ileostomy de reased to about 1 l/24 hours two dialyses per week were required Before the establishment of the ileostomy the serum level of creatinine averaged 15 6 mg of urea nitrogen 73 mg/100 ml and of potassium 6 6 mEq/l before dialysis After the operation the averages were 11 3 87, and 5 8 respectively Daily administration of purgatives to increase the excretion from the ileostomy was tried but was not tolerated by the patient who experienced nausea Resonium® (an ion exchange resin) 15 g was given daily larger doses caused vomiting

The patient s general condition deteriorated with the primary disease The hypertension heart volume and the fundus changes did not improve He lost weight by a further 6 kg in 6 weeks The course was complicated by pneumonia which was resistant to antimicrobic therapy The patient s catabolism continued with increasing cachexia Agonally the operation wound ruptured and the patient died on the 54th day after the establishment of the ileostomy

Autopsy showed subacute glomerulonephritis a greatly enlarged heart, extensive areas of pneumonia and peritonitis without any demonstrable focus

Case 2

A 34 year old accountant had since the age of seven attacks of intermittent convulsive pain in the abdomen, fever, elevated ESR and proteinuria He had undergone four operations for incipient intestinal obstruction with lysis of adhesions Amyloidosis was demonstrated by liver and kidney biopsy in 1964 He had increasing uraemia since 1964 but was nevertheless doing full time work

On admission to the medical clinic at Umea hospital in Nov 1965 he had generalized oedema and a B P of 200/120 mm Hg heart volume 500 ml/sqm serum creatinine 18 3 mg/100 ml rather ill defined papillae and some arterio-venous displacements in the eye fundi Intermittent haemodialysis was not considered suitable in this patien He was placed on a protein free diet supplemented with 10 g of amino acids in table form but could not stand it for more than three weeks Although it had been judged as unsuitable intermittent haemodialysis wa then considered in view of his age good working capacity well balanced psychic state and his will to live His first child had just been born The department s facilities at tha time allowed him only one haemodialysis per week He lived 350 km away from the hospital and wished to go home between the treatments As four initial dialyses failed to produce a satisfactory result, it was decided to perform ileostomy as a complement to the dialysis treatment

At operation one month after his admission to the hospital the ileum was divided 9 dm from the ileocecal valve and an ileostomy was made by Brooke s method The distal end was closed the proximal end was carried forwards as an ileostomy into the iliac fossa through the adominal wall

On the 18th post-operative day the patient complained of abdominal pain He was found to have a haematoma in the wound and showed signs of intraperitoneal haemorrhage At laparotomy about 1/2 l of blood was removed from the abdominal cavity

hoped that the excretion of water, electrolytes and, to some extent, nitrogenous products from the ileostomy would permit a reduction of the number of dialyses required and that the patients would thus be able to take more fluid and more salt rich food than before

The losses from the ileostomy amounted to about 800—2000 ml of water, 120—290 mEq of sodium 16—40 mEq of potassium 70—170 mEq of chlorides, 2—10 mEq of calcium 0 2—0 4 g of phosphorus 0 6—1 4 g of urea nitrogen and 0 1—0 3 g *of creatinine per* 24 hours The excretion from the ileostomy could replace the kidney function almost completely with respect to water, sodium calcium, and chlorides to the extent of 25—50 % with respect to potassium and phosphorus but only to 10—20 % with respect to nitrogen A diet low in nitrogen a 24 hour urinary output exceeding 1 l and or dialysis are required so as to achieve balance for nitrogen potassium and phosphorus in patients who suffer from chronic renal failure and in whom an ileostomy has been performed

Two factors reduced the value of ileostomy in our patients namely the excretion of fluid decreased after one and two months, respectively, to less than 1 l, and the excretion of potassium was not sufficient to allow a reduction of the number of dialyses for more than a short time The restrictions relating to the intake of salt and water could be eased

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Conjunctival Vascular Changes in Relation to Retinopathy and Nephropathy of Diabetes Mellitus

By

JORN DITZEL

Lundbæk (10) has provided reasons for regarding the various vascular anomalies in diabetes mellitus of long standing as various manifestations of one generalized vascular disorder, a diabetic angiopathy The most essential criteria that support this hypothesis are the following

1 The vascular disorder is located especially in the small arteries, arterioles capillaries, and venules and is histologically characterized by endothelial proliferation and the presence of increased amounts of PAS positive material in the vessel walls and their basement membranes

2 Clinically all the vascular abnormalities the morphologically specific (retinopathy, glomerulosclerosis) as well as the morphologically non-specific (coronary disease, occlusive vascular disease of the lower extremities) are recognized by features which include a close relationship between their occurrence and the duration of diabetes

3 In unselected cases of long standing diabetes the incidences of the specific and of the apparently non specific parts of the syndrome of long standing diabetes are united by a network of statistical correlations

Previous biomicroscopic studies (1, 3) have shown differences partly of a qualitative and partly of a quantitative nature between the conjunctival changes in diabetic and those in non diabetic subjects In the non diabetic subjects the conjunctival changes consist mainly of configurative irregularities in the arterioles and venules of venular sacculations and hyaline infiltration These changes rise in incidence with increasing age Changes of quite similar appearance are found among diabetics and these changes similarly become commoner with increasing age Besides this category of changes capillary elongation decreased A/V ratio and microscopic edema preponderate among the diabetics Although none of these changes

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hoped that the excretion of water electrolytes and, to some extent, nitrogenous products from the ileostomy would permit a reduction of the number of dialyses required and that the patients would thus be able to take more fluid and more salt rich food than before

The losses from the ileostomy amounted to about 800 —2000 ml of water 120—290 mEq of sodium 16—40 mEq of potassium 70—170 mEq of chlorides 2—10 mEq of calcium 0 2—0 4 g of phosphorus 0 6—1 4 g of urea nitrogen, and 0 1—0 3 g of creatinine per 24 hours The excretion from the ileostomy could replace the kidney function almost completely with respect to water sodium calcium and chlorides to the extent of 25 50 % with respect to potassium and phosphorus but only to 10 20 % with respect to nitrogen A diet low in nitrogen a 24 hour urinary output exceeding 1 l and or dialysis are required so as to achieve balance for nitrogen potassium and phosphorus in patients who suffer from chronic renal failure and in whom an ileostomy has been performed

Two factors reduced the value of ileostomy in our patients, namely the excretion of fluid decreased after one and two months, respectively, to less than 1 l and the excretion of potassium was not sufficient to allow a reduction of the number of dialyses for more than a short time The restrictions relating to the intake of salt and water could be eased

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		Group 2 (35) Without late Diabetic Manifestations	Group 5 (60) With late Diabetic Manifestations
VASCULAR CHANGES	ARTERIOLAR IRREGULARITIES	6 %	32 % *
	CAPILLARY ELONGATION	74 %	87 %
	VENULAR IRREGULARITIES	66 %	93 % *
	VENULAR SACCULATIONS	17 %	25 %
	ARTERIOLAR/VENULAR DIAMETER A/V RATIO 1:3	51 %	68 %
PERIVASCULAR CHANGES	EDEMA	26 %	72 % *
	HYALINE INFILTRATION	51 %	68 %
	HEMORRHAGES	6 %	3 %

Fig 1 The incidence of the various conjunctival changes among 60 diabetics with late diabetic manifestations as compared to the incidence among 35 diabetics of comparable age without retinopathy or nephropathy
* designates the finding of a significant difference in the incidence of a change between the two diabetic groups

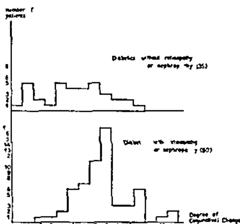


Fig 2 The degree of conjunctival changes in 60 diabetics with late diabetic vascular manifestations as compared to 35 diabetics without retinopathy or nephropathy

hyaline infiltration and hemorrhages in the conjunctival tissue

Information about the method for studying the conjunctival vessels and about the terminology used has been given previously (1, 2 4 7)

Results

Among the 60 diabetics with late diabetic vascular manifestations 34 had diabetic retinopathy without nephropathy Eighteen of these had simple retinopathy and 16 proliferative retinopathy Twenty six had both retinopathy and nephropathy Fifteen diabetics showed hypertension and all of these patients had nephropathy

Fig 1 shows the incidence of the individual types of change observed in the conjunctiva among the 60 diabetics with late diabetic vascular manifestations as compared to 35 diabetics without retinopathy and nephropathy These 35 diabetics consisted of the patients without late diabetic manifestations in group 2 (16—35 years) in a previously published study (1) It is seen from fig 1 that all types of change, with the exception of hemorrhages were found more frequently among the diabetics with late diabetic manifestations than among diabetics without retinopathy and nephropathy The difference in incidence is significant for the following changes arteriolar irregularities venular irregularities and edema ($p < 0.01$) The

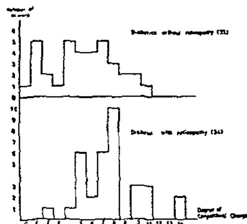


Fig 3 The degree of conjunctival changes in 34 diabetics with retinopathy as compared to 35 diabetics without late diabetic manifestations

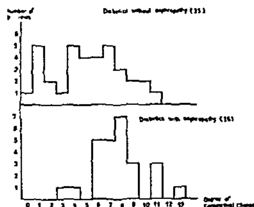


Fig 4 The degree of conjunctival changes in 26 diabetics with nephropathy as compared to 35 without diabetic manifestations

increased incidence of microscopic edema might have arisen solely from the presence of diabetic nephropathy. However the incidence of microscopic edema was 62 % among the 34 cases with retinopathy alone and 85 % among the 26 cases with nephropathy and this difference in incidence is not significant

In order to give a more lucid picture of the degree of conjunctival changes in relation to the presence of late diabetic vascular manifestations each change in the conjunctiva has been given points according to the impression of its severity. The following arbitrary point scale was used arteriolar irregularities venular sacculations and hyaline infiltration each 3 points hemorrhages and A V ratio 1 3 each 2 points capillary elongation venular irregularities and microscopic edema each 1 point If in one patient all these changes are present, it will give maximally 16 points

Employing this point scale, fig 2 shows the degree of conjunctival changes among the 60 diabetics with late diabetic vascular manifestations as compared to the 35 diabetics without retinopathy and nephropathy An X^2 test demonstrates that the group of patients with retinopathy or nephropathy significantly more frequent had more severe changes in the conjunctiva than diabetics without these vascular manifestations (X^2 = 7 88 n = 1, $p < 0.01$)

Fig 3 shows in a similar way the distribution of the degree of conjunctival changes in the 34 diabetics with retinopathy alone as compared to the 35 diabetics without late diabetic manifestations An X^2 test demonstrates that the diabetics with retinopathy alone have more severe conjunctival changes than the group of diabetics without retinopathy (X^2 = 6 17 n = 1 $0.01 < p$ 0 02

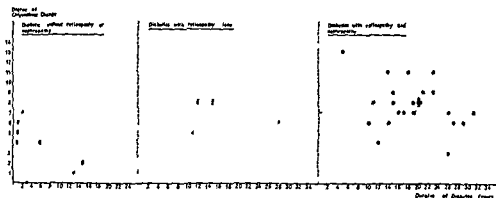


Fig 5 Corresponding degrees of conjunctival changes and duration of diabetes (see text)

Fig 4 shows likewise the degree of conjunctival changes in the 26 diabetics with nephropathy as compared to the 35 diabetics without late diabetic manifestations An χ^2 test shows that the diabetics with nephropathy have significantly severer conjunctival changes than patients without nephropathy ($\chi^2 = 6.10$ n = 1, $0.01 < p < 0.02$)

It could be objected that the relationship between the degree of conjunctival changes and the presence of retinopathy and nephropathy only reflected the common correlation of these manifestations to the duration of diabetes That this is not the case can be seen by fig 5, where corresponding degrees of conjunctival changes and duration of diabetes are indicated The coefficients of correlation for all three groups do not deviate significantly from zero

Discussion

This study indicates that there exists a significant correlation between the degree of conjunctival changes and the presence of retinopathy and nephropathy—a correlation which does not simply reflect a common dependence on the duration of diabetes In a brief congress communication Janert and Olbert (9) arrived at the same conclusion These investigators examined the conjunctival vessels in 225 diabetics and found, besides a correlation to the duration of diabetes also a correlation between more severe changes in the conjunctiva and the presence of retinopathy These correlations and the finding that the vessel walls in the conjunctiva of diabetics histologically show staining characteristics similar to the specific diabetic vascular manifestations, strongly support the hypothesis that the conjunctival changes are part of a diabetic angiopathy This conclusion adds to the significance of hemorheological investigations in young diabetics (5 6) in whom the bulbar conjunctiva was also the site studied These investigations suggest that prolonged venous stasis in the microcirculation, produced mainly by patho physiological functional charg

TABLE I Calculation of the volume of a r wh ch w th var ous distr bu ons of the vent lat on lung volumes and sp rometer volumes must be resp red in order to obta n a 90 % m x ng

Distr but on V_A of vent lation		V_S 5 000 ml		V_S 7 000 ml		$V_S = 9\,000$ ml	
		Resp vol 90 % (l)	f	Resp vol 90 % (l)	f	Resp vol 90 % (l)	f
F en	1 000	1 9		2 0		2 1	
	2 000	3 3	1 7	3 6	1 8	3 8	1 8
	4 000	5 2	1 6	6 0	1 7	6 5	1 7
	8 000	7 3	1 4	8 9	1 5	10 0	1 5
Uneven	1 000	6 5		6 7		7 0	
	2 000	11 2	1 7	11 9	1 8	12 8	1 8
	4 000	17 9	1 6	19 7	1 7	21 8	1 7
	8 000	26 1	1 5	30 4	1 5	33 6	1 5

V_A — lung volume
V_S sp rometer volume
Resp vol 90 % — the volume of a r measured n l tres) to be resp red to obta n a 90 % m x ng
f — resp vol 90 % at a g en lung volume d ded by resp vol 90 % at a lung volume half as large
Uneven vent lat on 75 % of the lung olume rece es 20 % of the en lat on e very une en ent lat on

Theoretical considerations

If the sp rometer volume lung volume and alveolar ventilat on are known it is possible to calculate the volumes of a r wh ch must be insp red (and exp red) in order to obta n a 90 % m x ng between the gas n the sp rometer and the lungs (10) Examples of such calculat ons are sho n in table I In some of the examples the vent lat on is assumed to be even in others it is assumed to be ery uneven so that 75 % of the lung volume rece ves 20 % only of the al eolar vent la t on

From the examples in wh ch the sp rom eter volume is assumed to be 7 000 ml and the vent lation is assumed to be even it w ll be seen that for lung volumes of 1 000 ml and 2 000 ml 2 0 and 3 6 l of a r respec t ely must be respired if a 90 % m x ng s to be obta ned The rat o bet een the t o volumes of a r respired is 1 1 8 wh ch is stated in column f of table I If the lung volume is 4 000 ml the necessary volume of resp red air is 1 7 t mes as large as that re qu red if the lung volume is 2 000 ml and consequently it is 1 7 × 1 8 times larger than that requ red if the lung volume were 1 000 ml For a lung volume of 8 000 ml the necessary volume of resp red a r is 1 8 × 1 7 1 5 t mes as large as that re quired for a lung volume of 1 000 ml

In the case of uneven vent lation the vol ume of gas to be resp red s larger than n even ventilat on but it st ll holds that rela ti e to th at for a lung volume of 1 000 ml 1 8 times as much a r must be resp red if t s 2 000 ml 1 8 1 7 t mes as much f it s 4 000 ml and 1 8 × 1 7 × 1 5 t mes as much f it s 8 000 ml

When exam n ng pat ents whether the ent lation s even or une en t s poss ble to calculate the volume of a r to be resp red f there s 90 % mixing w th a lung volume of only one l tre If the lung volume s 8 000 ml this calculat on is carr ed out by

dividing the volume of air respired by 1 8 × 1 7 × 1 5 If the lung volume is 4 000 ml the volume of air respired is divided by 1 8 × 1 7, and if the lung volume is 2 000 ml it is divided by 1 8 The conversion is carried out most easily on the basis of fig 1 The volume of respired air per litre of lung volume calculated in accordance with these principles increases with increasingly uneven ventilation In the following this volume of respired air will be termed the mixing efficiency

Since both the conversion factors and the necessary volume of respired air depend on the spirometer volume identical spirometer volumes must be applied in all the examinations However any errors arising out of variations of 2 000 ml in the spirometer volume will hardly exceed 20 % and errors of this magnitude will occur only if the lung volume exceeds 8 000 ml

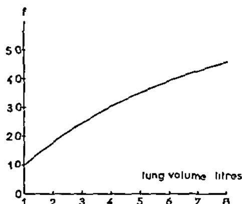


Fig 1 Mixing rate for various lung volumes f = the figure by which the volume of air respired to obtain 90 % mixing at a given volume must be divided in order to obtain the volume of air which is required for mixing if the lung volume is one litre

Method

A modified Krogh spirometer is used (10) Into the tubes there is inserted a blower which circulates the air at a high velocity so that the expired air can be reckoned completely mixed with the air of the spirometer before the beginning of the next inspiration The volume of the system (i e spirometer + blower + tubes) ranged between 6 000 and 7 000 ml (6 500 ± 500 ml) in all the experiments Thus the error arising out of variations in the volume of the system will not exceed 5 %

At the beginning of the examination the spirometer contains almost pure oxygen whereas the patient's lungs contain atmospheric air The nitrogen concentration in the spirometer is recorded continuously by means of a nitrogen meter the needle valve of which is inserted in the tubes The experiment is discontinued when the nitrogen concentration in the spirometer has remained constant for 30 sec The lung volume is calculated on the basis of the initial and final concentrations of nitrogen with corrections as previously stated (10) for the excretion from the tissues of 20 ml of nitrogen per minute — the maximum correction however being 140 ml

The period of time before 90 % mixing is obtained is read from the nitrogen meter recordings The volume of air respired during this period is read from a kymograph by means of which the respiratory movements are registered

From fig 1 is read the value f by which the volume of air respired must be divided in order to determine the mixing efficiency

Results and discussion

Altogether 54 examinations were carried out in 17 normal subjects (seven males and ten females, average age 47 years), and 79 examinations were carried out in 22 patients (15 males and seven females, average age 55 years) suffering from chronic bronchitis and emphysema From two to five measurements were made in each individual Table II presents examples of the results of the examinations In fig 2 the mean figures for each subject are plotted along the

TABLE II Examples of results obtained in normal subjects and in patients

No	Subject	$\frac{RV}{TC}$ 100 %	Vs	Resp vol 90 % (l)	FRC	f	ME	Mean
1	N	34	6 800	9 1	2,430	2 1	4 3	4 3
			6 700	7 8	2 300	2 0	3 9	
			6 800	9 ?	2 400	2 1	4 4	
			6 00	9 0	2 300	2 0	4 5	
?	N	3?	6 850	7 8	2 650	2 3	3 4	3 5
			6 450	9 0	2 500	2 2	4 1	
			6 350	6 6	2 400	2 1	3 1	
			6 450	6 4	2 250	2 0	3 2	
3	N	44	6 350	14 7	3 150	2 6	5 7	5 3
			6 350	10 6	3 000	? 5	4 2	
			6 500	15 0	3 000	2 5	6 0	
4	P	67	6 650	30 4	5 600	3 8	8 0	8 6
			6 850	35 2	5 530	3 8	9 3	
			6 600	31 6	5 550	3 8	8 5	
			6 00	34 8	5 960	4 0	8 7	
5	P	61	6 400	20 0	3 450	2 7	7 4	6 0
			6 100	18 0	3 350	2 7	6 7	
			6 400	13 7	3 100	2 6	5 3	
			6 500	12 1	2 960	? 4	5 0	
			6 350	13 3	2 900	2 4	5 5	
6	P	57	6 100	14 6	2 000	1 8	8 1	7 4
			6 300	12 8	2 100	1 9	6 7	
			5 950	12 6	2 000	1 8	7 0	
			6 100	14 3	1 940	1 8	7 9	

N normal subject
P = patient
RV = residual volume
TC = total capacity
Vs = spirometer volume
Resp vol 90 % the volume of air measured in l res to be respired to obtain a 90 % mixing
FRC functional residual capacity the lung volume at the end of a normal expiration
f the figure read from fig 1 by which resp vol 90 must be divided in order to determine the mixing efficiency
ME mixing efficiency

abscissa, while $\frac{RV}{TC} \times 100\,\%$ is plotted along the ordinate.

The normal value for the mixing efficiency was found to be $3.6 \pm 2 \times 0.7$ (mean $\pm$ 2 × SD). As appears from fig. 2, all patients except one presented a mixing efficiency above 5.0, whereas all normal subjects except one had a mixing efficiency below 5.0.

The inaccuracy of each individual measurement was found to be 0.5 and 0.9 for normal subjects and patients, respectively. In only five experiments was a normal (or abnormal respectively) mixing efficiency found in someone, in whom the total result of the examinations revealed an abnormal (or normal respectively) mixing efficiency. Consequently, only two measurements are required in each subject.

As will be seen from fig. 2, no conclusion can be reached as to the degree of uneven ventilation from the severity of the emphysema, evaluated on the basis of $\frac{RV}{TC} \times 100\,\%$. Hence, determination of the degree of uneven ventilation and assessment of the severity of the emphysema are complementary examinations.

It is frequently stated that an open method is to be preferred to a closed method in the demonstration of uneven ventilation because the open methods are more sensitive (6). However, this higher sensitivity is of importance only if small degrees of uneven ventilation are to be demonstrated, in which case it is necessary to know the terminal part of the nitrogen wash out curve and of the nitrogen mixing curve.

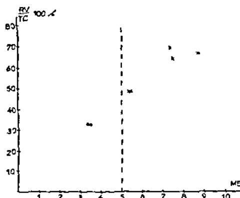


Fig. 2. $\frac{RV}{TC} \times 100\,\%$ in relation to the mixing efficiency (ME).
o = normal subjects. x = patients.

Hence the results obtained in the present study will challenge comparison with the results obtained by Strange Petersen (9), who used the open method, since the values revealed by the latter in normal subjects and patients were more prone to overlap than those found by the method now described.

Thus by applying our method, an estimation of the degree of uneven ventilation will readily be obtained simultaneously with a determination of the lung volume.

Summary

A new method of measuring the degree of uneven ventilation is described. The volume of air which must be respired in order to obtain a 90 % mixing between the air in the lungs and that in a spirometer is measured. From these values the mixing efficiency is calculated, i.e. the volume of air to be respired if the lung volume is not more than one litre.

Sixteen out of 17 normal subjects presented a mixing efficiency ranging below 5.0, whereas 21 out of 22 patients with chronic bronchitis and emphysema had a mixing efficiency of more than 5.0.

The severity of the emphysema, evaluated on the basis of $\frac{RV}{TC} \times 100\,\%$ provides no information as to the degree of uneven ventilation.

Acknowledgement

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Serum Insulin in Normal and Obese Persons

By

Torsten Deckert and Leif Hagerup

Since 1960 it has been possible to determine the insulin concentration in human blood by immunological methods These methods are distinguished from biological methods in being more sensitive, accurate and specific (3)

Whilst the serum level of insulin determined by immunological methods differs from that found by biological methods (14), there are also considerable variations in the immunologically determined fasting insulin concentration in normals

These variations are due to methodological and or immunological factors and to the non homogeneous nature of normal populations studied Concerning the methodological factors it need be mentioned only that they almost always lead to high values (7 11) whereas the immunological factors give low values (5) If the endogenous insulin to be determined reacts less to the insulin antibodies used in the method than does the standard insulin preparation the determined value will be too low There fore, the normal values found in various laboratories can be compared only when it has been verified that no difference exists between the immunological reaction of the endogenous insulin and that of the standard insulin preparation used However in most cases no verification has been done

Concerning the heterogeneity of published normal series, it should be noted that in most of them no regard has been paid to sex, age and body weight It would be useful to ascertain the importance of these factors

The purpose of the present study was to investigate some of these factors

Material and methods

Althogether 68 persons aged 49—51 years were investigated These persons were selected through the National Registry and requested to appear for an ambulatory check up Forty (19 women and 21 men) were of normal body weight while 28 (12 women and 16 men) were obese having a body weight exceeding the ideal weight by more than 15 % according to Natvig s tables (15

None had recognized diabetes mellitus pituitary disorder or fasting glycosuria and

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TABLE I Insulin concentration in non diabetic persons

Normal persons			Obese persons			
No of pats invest	Remarks	M±2 S D (μunits/ml)	No of pats invest	Remarks	M±2 S D (μunits/ml)	Authors
30		21±31				Yalow & Berson (1960)
30		23±35				Samols & Ryder (1961)
4	Arterial blood	9±6	6	Young persons	31±25	Rabinowitz & Zierler (1962)
25		58 (range 20—120)				Goetz et al (1963)
8		About 40	10		About 60	Karam et al (1963)
100		19±15				Samols & Marks (1963)
20	> 40 yrs old	61±45				Spellacy & Goetz (1963)
5	23—36 yrs old	17 (range 6—27)				Hales (1964)
11	Females 46—77 yrs old 64 % obese	20±20				Hales et al (1965)
75	Adults	9±9				Soeldner & Slone (1965)
11		8±16	3		29±36	Nikkila et al (1965)
45	Non obese 23—47 yrs old	9 (range 6—20)				Welborn et al (1966)
40	50 yrs old	28±10	28	50 yrs old	32±20	Deckert & Hagerup (1967)

all had a fasting blood sugar ⩽ 120 mg/100 ml Blood for insulin determination was drawn from the cubital vein after at least 8 hours starvation The serum was pipetted off and stored at —20 C Before the insulin determination 50 μl of heparin Leo (5 000 i u / ml) was added to each ml of serum to avoid incomplete precipitation of anti insulin (26) Two hundred μl of undiluted heparinized serum was used for each test

Insulin was determined by the duplicate antibody technique of Hales and Randle (7) as modified by Jorgensen (12) The principle of this analysis is that unlabelled insulin competes with labelled insulin for binding to insulin antibodies Since the quantity of

unlabelled insulin is the only variable component in the reaction mixture the amount of the radioactive insulin bound to the insulin antibodies indicates the concentration of unlabelled insulin present The insulin bound to the insulin antibodies is separated from the free insulin by adding anti gamma globulin to precipite the insulin insulin antibody complex. For practical reasons a few modifications were made All buffer solutions contained only 0 5 % human albumin and the incubation period for the reaction between insulin antibody and anti gamma globulin was only five hours while that for the reaction between the precipitate and insulin was 24 hours The radioactivity was measured in a γ spectrometer All determinations were made in duplicate The insulin antibodies preparation used was a serum from guinea pigs immunized by pig insulin with an insulin binding capacity of 1 2 i u/ml (4) it was diluted 1 8 000 The standard insulin used was a highly purified, re crystallized pig insulin (from the Steno Memorial Hospital Copenhagen) dissolved in 0 01 mole phosphate buffer pH 7 4 To avoid adsorption of insulin onto the glassware, the phosphate buffer contained 0 5 g of human albumin/100 ml (22) ^{131}I pig insulin was prepared as described by Hunter and Greenwood (10) (supplied by the Steno Memorial Hospital Copenhagen) Daily corrections were made for radioactive degradation products As anti γ globulin we used serum from rabbits immunized by a non purified guinea pig γ globulin preparation The anti γ globulin sera were made in Statens Seruminstitut Copenhagen Blood sugar was determined by reduction of potassium ferricyanide in an autoanalyzer

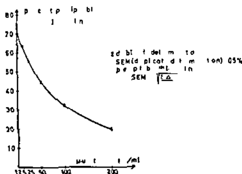


Fig 1 Standard curve produced by the addition of known amounts of unlabelled pig insulin

Results

Fig 1 shows a characteristic standard curve The accuracy of the method (M ± 2 S D), assessed by a 20 fold examination of the same serum on the same day was 21 ± 5 μunits/ml The reproducibility assessed by a 20 fold examination of another serum of ten different days using two different ^{131}I insulin preparations was of the same magnitude 29 2 ± 7 2 μunits/ml

On dilution of undiluted heparinized serum with 0 5 % human albumin buffer pH 7 4, to 1/2 1/4 1 8 and 1/16, no dilution effect was found (fig 2) Similar results were obtained with heparinized serum from obese persons (5)

Recovery of added insulin was 96 % when heparinized serum was diluted in the ratio 1 1 with a known amount of insulin whereas the recovery rate was 120 % when 50 μl of insulin solution was added to 500 μl of undiluted heparinized serum (tables II and III)

The distribution of the insulin concentration in heparinized sera from fasting normal weight persons is given in fig 3 The values are normally distributed There was no significant difference in insulin concentration between women and men ($0.2<p<0.3$)

The insulin concentration in most over weight patients was within the normal range However 25 % (seven

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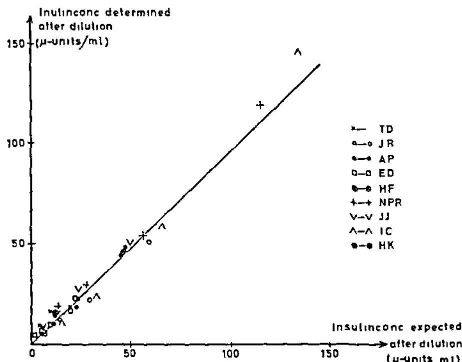


Fig 2 Serum insulin concentration in diluted serum from nine non diabetic patients (From Deckert and Jorgensen (5))

TABLE II Recovery of insulin in heparinized sera diluted 1 1 with 0 5 % buffered human albumin (pH 7 4) (μl units/ml)

No	Insulin conc of sera	Pig insulin added (μl units)	Insulin conc determined after adding insulin	Expected insulin conc	Recovery (%)
1	87	25	107	112	96
2	36	25	67	61	110
3	25	25	47	50	94
4	26	25	44	51	86
5	26	25	49	51	96
				Mean recovery 96 %	

out of 28 patients) showed a significant elevation of fasting insulin (more than 2 S D higher than the mean concentration in normal weight men and women (fig 3)

No correlation was found between the fasting insulin concentration and either the degree of overweight (fig 4) or the fasting blood sugar level (fig 5)

TABLE III Recovery of 50 μl of pig insulin in 500 μl of undiluted heparinized human sera (μl units/ml)

No	Insulin conc of sera	Pig insulin added (μl units)	Insulin conc determined after adding insulin	Expected insulin conc	Recovery (%)
1	19	36	61	55	111
2	25	39	73	64	114
3	38	37	86	75	115
4	15	36	59	51	116
5	24	39	73	63	116
6	18	36	64	54	118
7	30	39	82	69	119
8	18	36	65	54	120
9	16	36	63	52	121
10	29	37	80	66	121
11	28	37	80	65	123
12	36	37	90	73	123
13	28	39	84	67	125
14	25	39	80	64	125
15	25	37	80	62	129
				Mean recovery	120 %

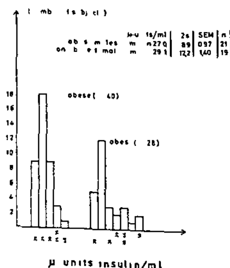


Fig 3 Insulin concentration of heparinized sera from 50 year old healthy persons

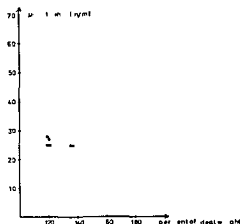


Fig 4 Correlation of insulin concentration and over weight

TABLE IV Blood sugar in different groups of non diabetic persons

Group	No invest	M[1]	S D	S E of mean	P[2]
Normal weight	40	99 1	9 7	1 53	~0 1
Obese	26	99 9	9 7	1 90	
Normal insulin concentration	58	98 2	9 3	1.22	<0 001
Elevated insulin concentration	8	108 1	8 1	3 06	

[1] M=mean blood-sugar concentration (mg/100 ml)
[2] P—probability according to Student s t test

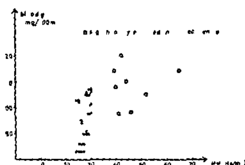


Fig 5 Correlation of insulin and blood sugar concentrations

There was likewise no difference in fasting blood sugar level between normal and over weight persons but there was a significant difference in blood sugar level between persons with a normal and persons with an elevated insulin concentration (fig 5 table IV) There was no correlation between insulin concentration and either the blood pressure or a family history of diabetes

Discussion

As discussed elsewhere (3) it is apparent from the dilution experiments that by the method used there is no demonstrable immunological difference between the circulating endogenous insulin and the standard insulin preparation used in this case pig insulin Evidently, the values found for the insulin concentration can be considered real genuine

The reason for the high recovery in undiluted serum is unknown The experimental result indicates that the radioactivity in the precipitate was too low possibly due to a slight pH difference between the undiluted serum and the buffered serum dilution this difference might conceivably lead to incomplete precipitation of the insulin antibodies

It is apparent from fig 3 that the insulin concentration in normal weight persons aged 50 years is distributed normally around 28 μunits insulin ml whereas over weight persons did not show a normal distribution neither for μunits ml nor for log μunits ml In seven of the 28 obese persons the insulin concentration was significantly higher than in normals Other workers have also observed a high insulin concentration in some fasting over weight non diabetic persons (table I)

None of the persons having a raised serum insulin level showed significantly elevated blood sugar but the group as a whole had a significantly increased mean blood sugar concentration Blood sugar regulation in these persons is therefore disturbed The pattern matches that seen in over weight diabetics (1), and it seems reasonable to assume that these are the persons who later develop dia betes mellitus

It is not yet known which patho physiological mechanisms cause this abnormal pattern One factor is that the muscle tissue of obese and 'stable onset diabetics becomes less sensitive to insulin (2) However, this factor is probably not of genetic nature as the insulin sen sitivity can be to some extent re established by weight reduction (18)

Summary

The mean fasting insulin concentration in heparinized sera from normal weight healthy persons aged 50 was 28 μunits insulin ml determined in duplicate by an immunological antibody technique There was no difference in concentration be tween men and women About 25 % of healthy over weight persons aged 50 showed elevated insulin levels in heparinized serum The cause is unknown None of the persons having an elevated insulin concentration showed significant ly increased blood sugar, but the group as a whole had a significantly increased mean blood sugar level It is concluded that persons with an increased fasting insulin concentration presumably have a disturbed blood sugar regulation even though the fasting blood ugar concen tration is within the normal range

Acknowledgements

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Glucose Tolerance and Serum Lipid Levels in Patients with Cerebrovascular Disease

By

THEODOR JAKOBSON

The status of carbohydrate metabolism in cerebrovascular disease has not been clear While some post mortem studies indicate that diabetes is associated with an increase in the frequency and severity of cerebral atherosclerosis (2 11), other authors feel that the incidence of cerebrovascular accidents is no higher in diabetics than in non diabetic persons (21, 26) On the other hand there have been only few studies of carbohydrate metabolism in patients with clinical evidence of cerebrovascular disease

In recent years the concept of the so called prediabetic state has emerged which may be defined as the state of those individuals who will eventually develop diabetes but in whom no abnormality of carbohydrate metabolism can yet be detected (7) The prediabetic state is followed by a preclinical diabetic state in which abnormalities of glucose tolerance can already be found by means of a standard glucose tolerance test (GTT) or by means of more sensitive steroid glucose tolerance tests which were first introduced by Fajans and Conn (9) More evidence has been gained that different diabetic manifestations including both diabetic micro angiopathy and involvement of the larger blood vessels can precede clear cut clinical diabetes One may therefore inquire whether there can also be found an association between cerebrovascular disease and preclinical diabetes which can be detected by testing glucose tolerance

Evidence has recently been accumulating on the surprisingly high incidence of abnormal glucose tolerance tests in other forms of vascular disease such as coronary heart disease (1 4 5 10 13 19 22 25) or peripheral arterial disease (5, 13 23) There has been discussion of the correlation of the observed disturbances of carbohydrate metabolism to increased serum lipid levels as well as of the significance of these metabolic alterations in the pathogenesis of occlusive arterial disease

Because of these findings it was considered worth while to examine glucose tolerance by means of a standard oral

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GTT test and a prednisone glucose-tolerance test (PGTT) in non diabetic patients with arteriographically confirmed atherosclerotic involvement of the cerebral vessels and in control subjects without evidence of cerebrovascular disease Determinations of serum cholesterol and serum triglycerides were carried out in these subjects at the same time, and an effort was made to correlate the results of the glucose tolerance tests with the blood levels of the circulating serum lipids

Material and methods

The material consists of 52 patients (41 males and 11 females) with cerebrovascular disease who were hospitalized at the neurological department of Hesperia Hospital in Helsinki The mean age of the men was 54 9 years range 36 to 65 years) and that of the women 51 4 years (range 42 to 61 years) In addition to a complete clinical neurological examination carotid and/or vertebral angiography was performed in a total of 47 patients In the remaining five cases the diagnosis of cerebral thrombosis was established on clinical grounds alone Complete occlusion of major cerebral arteries was found on arteriographic examination in 18 cases marked stenosis was present in 12 patients and in 17 there were arteriosclerotic changes of a lesser degree or disease predominantly affecting small vessels The following categories of patients were exluded from the material those aged over 65 those in whom the diagnosis of cerebrovascular disease was considered to be uncertain, those with overt diabetes and markedly obese subjects Altogether 29 patients with cerebrovascular disease had simultaneous cardiac involvement Clinical and electrocardiographic evidence of coronary heart disease was present in seven cases electrocardiographic or roentgenological signs of arteriosclerotic heart disease in eight cases, and signs of hypertensive heart disease in 14 cases Four additional patients had persistent elevation of blood pressure above 160/100 mm Hg without cardiac involvement

A family history of cerebrovascular accident was obtained in nine patients and a family history of probable coronary heart disease in altogether 19 cases The occurrence of vascular complications in close relatives (parents or siblings) of patients with cerebrovascular disease was found to be more pronounced in those patients who in addition to the involvement of the cerebral arteries had signs of coronary or arteriosclerotic heart disease (16 out of 29 patients) than in patients without clinical evidence of cardiac involvement (eight out of 23 patients) A family history of diabetes mellitus was obtained in five additional cases

Glucose tolerance was examined in patients with cerebrovascular disease during the period *of convalescence, on an average 4—6* weeks following the acute episode, either by means of a standard glucose tolerance test (28 cases) a prednisone glucose tolerance test (38 cases) or both (13 cases)

Adequacy of carbohydrate intake was ensured at least three days prior to the glucose-tolerance tests which were performed by administering after an overnight fast 1 g of glucose per kg of body weight as a 20 % solution Capillary blood was drawn for the determination of blood glucose prior to the administration of the glucose load and thereafter at 1/2 hour intervals for three hours The determinations of blood glucose were performed with the aid of an Auto Analyzer® by a specific enzymatic procedure based on the use of glucose oxidase (15)

The prednisone glucose tolerance test PGTT) was performed according to the original cortisone glucose tolerance test described by Fajans and Conn (9) Instead of cortisone equivalent amounts of prednisone were administered to the patients Persons weighing less than 72 5 kg (160 lb) thus received 10 mg of prednisone and persons weighing more than 72 5 kg received 12 5 mg of prednisone 8 1/2 hours and again two hours before the administration of the glucose load Blood samples for the determina

tion of blood glucose were drawn just as in the standard oral glucose tolerance test. A glucose tolerance test was as specified by Fajans and Conn regarded as positive if the 1 hour blood glucose value exceeded 160 mg % and the 2 hour blood-glucose value exceeded 120 mg %. The criteria for a positive prednisone glucose tolerance test were a blood glucose value above 160 mg % one hour following the glucose load and a blood-glucose value above 140 mg % two hours after the glucose load. Glucose tolerance curves which did not meet these criteria but which showed markedly elevated peak values often accompanied by glucosuria were defined as borderline tests.

Glucose tolerance tests were similarly performed in 21 age matched control patients without cardiac involvement who had been admitted to the hospital for the evaluation of symptoms caused by non vascular nerological disorders. The absence of atherosclerotic involvement of the cerebral arteries was in these subjects demonstrated by means of arteriography. Glucose tolerance was in addition estimated in 30 non-obese hospitalized patients with minor neuropsychiatric disturbances who did not show any clinical evidence of cerebrovascular or cardiovascular disease. The mean age of the whole control group was 50.2 years with an age distribution (36 to 65 years) similar to that in the patients with cerebrovascular diease. A total of 29 glucose tolerance tests and 26 prednisone glucose tolerance tests were performed in the control subjects.

Determinations of serum cholesterol were performed in the patients with cerebrovascular diease and in the control group according to Pearson et al (18). Three to five blood samples were obtained from each patient and the recorded serum-cholesterol values represent on an average mean values of 3.3 determinations. Serum triglycerides were determined according to Carlson and Wadstrom (6).

Results

The results of the glucose tolerance tests and the prednisone glucose tolerance tests obtained in patients with cerebrovascular disease and in control subjects with no evidence of cerebrovascular disease are shown in table I. Absence of cerebrovascular disease was shown in the

TABLE I. Results of glucose tolerance tests in patients with cerebrovascular disease and in control subjects without clinical evidence of cerebrovascular involvement

	No of pats	Mean age (yrs)	Positive tests	Borderline tests	Negative tests
Oral glucose tolerance test					
Cerebrovascular disease	28	54.4	6 (21.4 %)	3 (10.7 %)	19 (67.9 %)
Control subjects	29	48.4	3 (10.3 %)	2 (6.9 %)	24 (82.8 %)
Prednisone glucose tolerance test					
Cerebrovascular disease	38	54.0	19 (50 %)	9 (23.7 %)	10 (26.3 %)
Control subjects	26	51.9	10 (38.5 %)	5 (19.2 %)	11 (42.3 %)

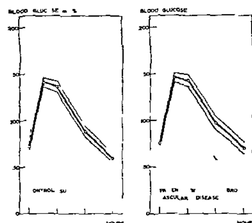


Fig 1 Mean glucose tolerance curves obtained in control subjects and in patients with cerebrovascular disease following an oral glucose tolerance test The shaded area represents the standard error of the means Standard deviations are shown by broken lines

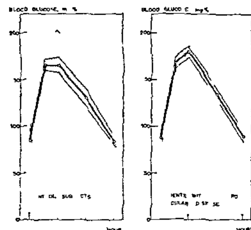


Fig 2 Mean glucose tolerance curves obtained in control subjects and in patients with cerebrovascular disease following a prednisone glucose tolerance test The shaded area represents the standard error of the means Standard deviations are shown by broken lines

control group by means of neurological examination moreover atherosclerotic involvement of the cerebral vessels was excluded by means of arteriography in eight subjects belonging to the GTT group and in 13 control subjects in whom the PGTT test was performed It can be seen that whereas the percentage of positive glucose tolerance tests in the patients with cerebrovascular disease was 21 4 % a total of 50 % of the patients had positive PGTT tests The corre

TABLE II Comparison of glucose tolerance in patients with various degrees of atherosclerotic cerebrovascular disease The mean values represent changes in the blood glucose con

	No of pats	Mean age (yrs)		Oral glucose tolerance test (hrs) 0	1/2	1	2	3
Complete occlusion of major cerebral arteries / Stenosis or marked atherosclerotic alterations	14	54 6	Mean	77 7	+74 6	+71 5	+ 18 5	0[2]
			S D	12 4	22 1	29 2	36 2	24 4
Atherosclerotic involvement of a lesser degree	9	51 6	Mean	70 3	+65 7	+59 9	+12 2	−18 6
			S D	11 1	22 8	23 3	13 8	19 8
Control subjects without arteriographic alterations of cerebral vessels	8	48 5	Mean	68 1	+57 8	+44 6	+ 4 1	−21 5
			S D	8 0	27 8	17 0	19 2	12 8

[1] P<0 05 when compared to control group
[2] P<0 02 when compared to control group

sponding figures for the control group were 10 3 % and 38 5 % respectively

Figs 1 and 2 show respectively the mean glucose tolerance curves and the mean prednisone glucose tolerance curves obtained in cerebral atherosclerotics and in the control subjects The mean glucose tolerance curve for the patients with cerebrovascular disease was not significantly different from that for the control subjects The augmented prednisone test was found to give somewhat higher values in cerebral atherosclerosis than in the controls, and the peak of the curve was observed at one hour instead of half an hour Here again the difference between the two groups was not statistically significant

Glucose tolerance tests on patients who on angiographic examination, showed either complete occlusion of major cerebral vessels or various degrees of atherosclerotic involvement have in table II been compared with similar tests performed in control subjects with arteriographically demonstrated absence of cerebrovascular abnormalities

Results obtained with the two types of glucose tolerance tests in patients with and without elevated serum cholesterol (>2 8 g/l) and/or triglyceride values (>1 6 mM) are compared in table III Patients with both kinds of serum lipid alterations were included in the same group because most hypercholesterolemic patients were found to have at the same time increased serum triglyceride levels while only a few patients had hypertriglyceridemia alone Statistically significant differences in blood glucose concentration between the two groups of patients were observed during the prednisone glucose tolerance test at two hours and at three hours

A comparison of glucose tolerance in 15 patients who in addition to cerebro-

involvement of the cerebral arteries and in control subjects without arteriographic signs of centrations (mg %) following the administration of the glucose load

No of pats	Mean age (yrs)	Prednisone glucose tolerance test (hrs)					
			0	1 2	1	2	3
11	53 5	Mean	85 2	+82 4	+87 0	+43 6	+ 5 5
		S D	8 1	31 3	34 2	27 1	27 5
10	56 8	Mean	87 9	+75 7	89 2	+58 4	−10 3
		S D	13 8	30 9	35 5	35 4	41 3
13	53 7	Mean	87 6	−75 0	+87 8	+35 2	—10.0
		S D	8 2	29 8	33 9	20 1	19 9
13	52 0	Mean	83 3	+72 9	−74 5	34 1	+ 7 1
		S D	10 9	20 8	39 5	41 7	20 9

TABLE III Comparison of glucose tolerance in patients with cerebrovascular disease who had mean values represent changes of blood glucose concentrations (mg %) following

	No of pats	Mean age (yrs)	Glucose tolerance test (hrs)	0	1/2	1	2	3
Patients with elevated serum cholesterol and/or triglyceride levels	16	51 0	Mean	78 1	+70 9	+70 3	+22 0	—6 2
			S D	11 5	24 7	27 5	36 5	26 6
Patients with normal serum cholesterol and triglyceride levels	11	55 7	Mean	70 6	+71 1	63 8	21 4	—3 6
			S D	11 0	15 8	25 3	35 5	26 7
			P					

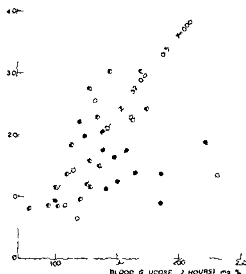


Fig 3 Correlation between serum triglyceride levels and 2 hour blood glucose values during a prednisone glucose tolerance test Control subjects without cerebrovascular alterations are shown by open circles patients with cerebrovascular disease and additional cardiac involvement by filled circles and patients with cerebrovascular disease who did not show any clinical signs of cardiac disease by half filled circles The equation represents the last mentioned group of patients

vascular disease, had evidence of coronary or arteriosclerotic heart disease and cerebral atherosclerotics without cardiac involvement failed to show any statistically significant difference between the two groups, although a slight tendency towards impaired glucose tolerance could be observed in the former group of patients Glucose tolerance of hypertensive patients with cerebrovascular disease was likewise not significantly different from that in normotensive patients

The mean serum cholesterol level of the patients with cerebrovascular disease was found to be elevated (3 1 g l) but only slightly above that in the control subjects with arteriographically confirmed absence of cerebrovascular involvement (2 9 g l) The difference between the two groups was not statistically significant The mean serum triglyceride levels of the two groups 1 65 mM and 1 52 mM respectively were likewise not significantly different Serum cholesterol or triglyceride levels of men and women with cerebrovascular disease were also found to be similar, and the serum lipid levels were not

elevated serum cholesterol or triglyceride levels and in patients with normal serum lipids The the administration of the glucose load

No of pats	Mean age (yrs)	Prednisone glucose tolerance test (hrs)					
			0	1/2	1	2	3
21	54 8	Mean	86 6	+81 1	+91 1	+55 1	+10 8
		S D	11 0	26 4	28 9	28 4	33 0
11	55 6	Mean	88 7	+79 7	+93 4	−34 2	−14 6
		S D	7 4	34 3	33 9	24 0	14 1
		P				<0 05	<0 01

markedly influenced by the age of the patients A grouping of the cerebral atherosclerotics into different categories according to the presence or absence of cardiac involvement or hypertension, and according to severity of cerebro vascular involvement, likewise failed to reveal any significant differences in the blood levels of cholesterol or triglycerides

The triglyceride values measured in patients with atherosclerotic involvement of the cerebral arteries, and in control subjects lacking arteriographic alterations of cerebral vessels, have in fig 3 been plotted against the 2 hour blood glucose values obtained in the same subjects fol lowing the prednisone glucose tolerance test A highly significant correlation ($P<0\,001$) was found to exist between the 2 hour blood glucose levels and the serum triglyceride levels of those patients with cerebrovascular disease who did not have additional cardiac involvement whereas the correlation between the blood glucose values and the triglyceride levels of the patients with additional cardiac involvement was less pro nounced No correlation could be ob served between the serum cholesterol levels and any of the blood glucose values

Discussion

Compared with that in previous reports, the impairment of glucose tolerance ob served during the course of the present investigation is perhaps less pronounced than, for example, in the series of Bohle and Schrade (5) who, with a different type of glucose tolerance test (Staub Traugott test) found decreased carbo-hydrate tolerance in 39 % out of 38 pa-tients with cerebral atherosclerosis or in the patients of Justin Besançon et al (13) who found decreased glucose toler ance in a total of 24 patients (89 %) out of 27 survivors of cerebrovascular ac cidents caused by cerebral thrombosis These figures are however not directly comparable with the results of the pres ent study e g because the mean age of the latter group was over 65 years and carbohydrate tolerance was not in vestigated in control subjects with a similar age distribution Perhaps the age factor likewise did not receive adequate

attention in the evaluation of the results of glucose tolerance tests in some of the previously mentioned studies performed in patients with coronary heart disease Possibly the observed high percentage of abnormal glucose tolerance tests might be partly explained by the well known 'dysglycemic' blood glucose response which is frequently encountered in old age (3, 24) and which has so far not been satisfactorily explained, although according to recent reports this phenomenon might be related to diminished insulin secretory capacity of the pancreatic islands in the elderly (8)

The significance of the impairment of glucose tolerance observed in patients with various clinical manifestations caused by atherosclerotic involvement of the arteries has in several reports been further obscured by the lack of an adequate control material During the present study an attempt was made to confirm the absence of cerebrovascular lesions in the control subjects by means of arteriography The obvious difficulties in excluding from the control group, on a clinical basis subjects with latent atherosclerotic involvement of other parts of the vascular system must however, be taken into consideration in comparing glucose tolerance in cerebral atherosclerotics with that in the subjects without clinical evidence of cerebrovascular or cardiovascular disease in whom impairment of glucose tolerance was also frequently observed

The mean serum cholesterol levels of patients with cerebrovascular disease were during the course of the present study found to be somewhat elevated although not significantly different from those in the control group, while the mean serum triglyceride concentrations were only slightly above normal Previous observations on serum lipid concentrations in cerebrovascular disease have yielded somewhat conflicting results Thus in a group consisting of 68 male patients with cerebral infarction caused by atherosclerosis, Heyman et al (12) reported significantly higher serum cholesterol levels than in patients with non atherosclerotic illnesses and Robinson et al (21) noted higher beta lipoprotein cholesterol concentrations and beta alpha lipoprotein ratios in patients with cerebral thrombosis than in age matched controls, while other studies have failed to demonstrate a significant elevation of serum cholesterol in cerebral thrombosis (16) On the other hand, significantly higher triglyceride concentrations have been reported in patients with cerebrovascular disease than in normal control subjects (14) The reasons for the discrepancies in the results reported by various workers are not readily apparent, although differences in the age and sex of the patients or in the severity of the atherosclerotic involvement probably should be considered, besides a lack of uniformity in the selection of control groups

In general, no correlation between serum cholesterol and impairment of glucose tolerance has been found in patients with coronary heart disease (1, 19, 22 25), although survivors of myocardial infarction with an abnormal glucose tolerance test have been reported to have somewhat higher mean serum triglyceride concentrations than patients without impairment of glucose

tolerance (19). The slight impairment of glucose tolerance which during the course of the present study was observed in those patients with cerebrovascular disease who at the same time had elevated serum cholesterol or triglyceride levels is therefore of interest although these observations allow of no conclusions concerning a possible causal relationship between the derangements of carbohydrate and lipid metabolism.

It is still questionable whether a pathogenetic role similar to that suggested for the more frequent disturbances of carbohydrate metabolism reported in patients with coronary heart disease can be ascribed to the slight metabolic alterations observed in cerebral atherosclerotics. An additional mean for probing the causation of deficient utilization of glucose and the significance of associated alterations of serum lipids in patients with various clinical manifestations of atherosclerosis is possibly provided by assay for plasma insulin. Thus an abnormally high response of plasma insulin as determined by immuno assay was recently observed in 55 % of survivors of myocardial infarction while no correlation was observed between plasma insulin and serum lipid levels (17). Similar studies in patients with cerebrovascular disease might throw additional light on the significance of metabolic alterations in patients with occlusive arterial disease.

Summary

Glucose tolerance was examined by means of an oral glucose tolerance test (GTT) or a prednisone glucose tolerance test (PGTT) in 52 patients with cerebrovascular disease and in age matched patients with minor neuropsychiatric disturbances who did not show any clinical signs of cerebrovascular or cardiovascular disease. A positive GTT was obtained in six out of 28 patients with cerebrovascular disease (21.4 %) and a positive PGTT in 19 out of 38 patients (50 %) although the mean blood glucose concentrations following either type of glucose tolerance tests were not found to be significantly higher than in the control subjects. Patients who on arteriographic examination were found to have complete occlusion or marked stenosis of major cerebral arteries showed a tendency towards a decrease of glucose tolerance when compared with patients having lesser degrees of atherosclerotic involvement or with control subjects without arteriographic alterations of the cerebral vessels.

Glucose tolerance was found to be somewhat decreased in those patients with cerebrovascular disease who had elevated serum cholesterol or triglyceride levels when compared with patients having serum lipid levels within the normal range. A significant correlation was found to exist between serum triglyceride levels of patients with cerebrovascular involvement and the 2 hour blood glucose values measured in the same patients during the PGTT test. No association could on the other hand be observed in cerebral atherosclerotics between the serum cholesterol levels and glucose tolerance.

It is concluded that the observed impairment of glucose tolerance in patients with cerebrovascular disease seems to be

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Long-term Heparin Treatment in Ischaemic Heart Disease

Effects on clinical condition and plasma lipids

By

L E Bottiger, L A Carlson, L Engstedt and L Oro

In 1956 Engelberg and co workers reported a study on the effect of intermittent heparin therapy on the clinical course of human coronary atherosclerosis (9) They had four cardiovascular deaths over a 2 year period in 105 patients given 200 mg of heparin subcutaneously twice a week as compared to 21 cardiovascular deaths in a control group of 117 patients Against this background we decided in 1957 to study the effect of such treatment in patients with ischaemic heart disease The main purpose was to follow the clinical course and the plasma lipid levels It was considered that the effects on plasma lipid levels were of special interest as no studies of larger groups were available neither on the effect of chronic heparin therapy nor on the spontaneous variations during prolonged periods

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Material

The study was planned and started in 1957 by Bottiger Carlson and Engstedt who also jointly did the clinical part during the entire study Dr Oro joined the group and participated in the clinical part from 1961 Dr L Sundell took active part in the work during 1958 to 1960

The material was selected in the following manner All records for men from the years 1952—1957 from the Department of Medicine Karolinska Sjukhuset with the diagnoses of myocardial infarction cardiosclerosis and/or angina pectoris (corresponding to the WHO classification numbers 420 and 422) were scrutinized As it was found that the number of patients eligible for the study was small the perusal of charts was extended to the out patient department of the same hospital as well as to the Department of Medicine Danderyd Hospital Dr G Berg Head of the Department of Medicine Danderyd Hospital kindly allowed us to include patients from his department in the study The latter is situated close to Stockholm and cares for patients from

infarction or onset of angina The stratification groups were

Age	Duration of disease	
	<5 years	>5 years
<45	A 1	A 2
46—55	B 1	B 2
56—65	C 1	C 2

For each of the six stratification groups an equal number of lots carrying the figures I or II respectively were used In order constantly to keep the treatment groups equal in size only a small number of lots were used within each stratification group from the beginning and a new series added when the first was finished The patient was given a group label according to the stratification (A 1—C 2) at the time the decision was made to accept him The nurse giving the injections then drew a lot for the patient and according to its figure enlisted him in treatment group I or II

Fasting blood samples were withdrawn on three consecutive days for determination of plasma lipids on the first day immediately before the injection and on the second and third days 24 and 48 hours after the injection

On all control examinations the patients were given standardized questions as to their opinion of the treatment and its effect ECGs were taken as initially both at rest and after working test and the physical working capacity again determined

Patients who developed a non fatal myocardial infarction during the study were hospitalized and given conventional treatment including anticoagulants (dicoumarol) They were afterwards allowed to continue the long term treatment but only *one incident per patient was taken into account for this study*

After the first infarction during the treatment period the patient was excluded from the controlled trial

Treatment schedule

The patients were given subcutaneous injections twice weekly in the anterior aspect of the thigh At each occasion 0 8 ml was given of preparation I or II both supplied by AB Vitrum Stockholm One contained 250 mg of heparin (25 000 units) per ml the other a coloured placebo fluid The code was kept by AB Vitrum and not broken until after the completion of the study *The study was finished when the statistician reported that significant differences were found between the number of incidents in the two groups*

All injections were given at the out patient department by a special nurse Conventional treatment with vasodilator drugs — nitrate and aminophyllin preparations — and sedatives were given when necessary All patients were instructed to keep an ordinary diet None received oral anticoagulants

The patients were called upon for control examinations after six months of treatment and then once yearly Physical examination and routine laboratory tests were performed

Methods

Plasma lipid analysis Blood was withdrawn from an antecubital vein in the mornings after fasting over night About 10 ml of blood was taken into a tube containing 5 mg of heparin Plasma was separated off by centrifugation and immediately extracted Plasma lipids were determined as described earlier (4 5) cholesterol according to Sperry Webb and triglycerides by analysis of triglyceride glycerol During the six years the study has been in progress different batches of standard cholesterol and tripalmitin as well as standard plasma have been used All new standards have been carefully checked against the previous ones

Lipoprotein lipase activity in plasma after the s c injection in the fasting state of 200 mg of heparin was estimated in 23 patients Free fatty acids released during incubation of the blood plasma samples with a fat emulsion (Intralipid[R] AB Vitrum Stockholm) in the presence of albumin were titrated according to the method of Boberg and Carlson (2)

Coagulation time was measured (in glass tubes) in 12 patients according to a slight modification of the Hedenius method (12), 3 6 8 and 24 hours after a heparin injection The coagulation analyses were performed at the Wallenberg Coagulation Research Laboratory Karolinska Institutet (B Blomback)

Subjective effect On each control examination the patients were given standardized questions regarding their subjective impressions of the effect of the treatment (How do you feel now? Do you feel better, the same or worse compared to before the treatment?) The answers were recorded literally and were later classed in five categories
Definitely better
Probably better
No effect
Probably worse
Definitely worse

The number of patients in each category was registered after 1/2 1, 2 3 etc years and the treatment effect measured as the percentage of patients reporting that they were definitely or probably better

Statistical methods

The significance of the difference between the number of incidents in the two treatment groups was calculated by means of the following modified t test which corrects for the continuous decrease in the number of patients

n_i = number of patients at the beginning of year i
d_i = number of incidents during year i
a_i — number of patients disappearing from the trial for other reasons during year i
Thus $n_{i+1} = n_i - a_i - d_i$

The probability, p_i for an incident to occur during year i

$$p_i = \frac{d_i}{n_i - \frac{a_i}{2}}$$

The probability P_i, for an incident to take place within i years is then

$$P_i = 1 - (1 - p_1)(1 - p_2) \ldots (1 - p_i)$$

An approximate estimate of the standard error of P_i σ_{P_i} is calculated according to

$$\sigma_{P_i} \approx (1 - P_i) \sqrt{\frac{d_1}{\left(n_1 - \frac{a_1}{2}\right)\left(n_1 - \frac{a_1}{2} - d_1\right)} + \frac{d_2}{\left(n_2 - \frac{a_2}{2}\right)\left(n_2 - \frac{a_2}{2} - d_2\right)} + \ldots + \frac{d_i}{\left(n_i - \frac{a_i}{2}\right)\left(n_i - \frac{a_i}{2} - d_i\right)}}$$

The standard error of the difference between the number of incidents in groups I and II $P_I - P_{II}$, is then

$$\sigma_{P_I - P_{II}} = \sqrt{\sigma_{P_I}^2 + \sigma_{P_{II}}^2}$$

The hypothesis that P_I and P_{II} are similar is tested according to

$$t = \frac{P_I - P_{II}}{\sigma_{P_I - P_{II}}} \quad (\text{d f } \infty)$$

Results

The groups resulting from the stratification and random allocation were homogeneous, not only with regard to the stratification factors age and duration (fig 1, table II) of disease, but also with regard to initial body weight (fig 1, table II), plasma lipid levels (table IV), and type of ischaemic disease (table II)

A Clinical results

1 Myocardial infarctions and sudden deaths

During the entire trial there occurred in the heparin group eight incidents — seven myocardial infarctions and one sudden death — as compared to 16 — ten infarctions and six sudden deaths — in the placebo group (table III) Fig 2 shows that during the first year the number of incidents are similar in both groups Later the total number of incidents is lower in the heparin group

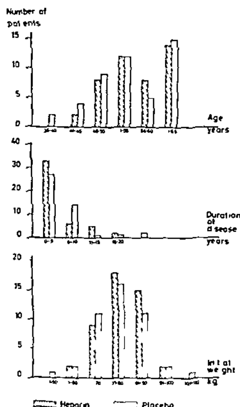


Fig 1 Distribution of the patients in the two groups with regard to age duration of ischaemic disease and body weight

TABLE II The composition of the final groups at the beginning of the treatment Mean values ± SEM and (below) standard deviation

Group	Heparin n = 46	Placebo n = 45
Age at start of treatment (yrs)	54 9 ± 1 0 7 1	55 0 ± 1 0 6 8
Duration of disease (yrs)	5 1 ± 0 7 4 5	5 3 ± 0 8 5 5
Initial weight (kg)	76 5 ± 1 3 9 0	75 2 ± 1 7 11 0
Type of initial disease (nos)		
Angina pectoris	24	24
Myocardial infarction	22	21

TABLE III Number of incidents (definite myocardial infarction or sudden death) in the two groups during treatment Only one incident per patient is included in the table After the occurrence of a non fatal incident the treatment was continued but the patient excluded from the controlled clinical trial

Incidents	Treatment (yrs) 1	2	3	4	5	6
No each year						
Heparin	5	2	0	0	1	0
Placebo	4	6	4	0	1	1
Cumulative figure at the end of the year						
Heparin	5	7	7	7	8	8
Placebo	4	10	14	14	15	16
No of pats at the beginning of the year						
Heparin	46	39	35	35	31	15
Placebo	45	40	33	27	22	8

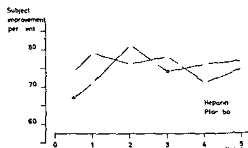


Fig 3 Percentage of patients in the two groups reporting subjective improvement during the treatment at the standardized control examinations

statistically significant at the one per cent level It turned out, however, that the statistical formula used by the statistician was erroneous (7)

2 Subjective improvement

Approximately 75 % of the patients reported that they were definitely or probably improved by the treatment as seen in fig 3 The figures are identical for both groups and they are constant during the whole study One patient in each group stated that he was definitely worse, the rest that no change had occurred

3 Side effects

No significant side effects were observed No allergic manifestations occurred nor any bleeding episodes In a few instances minor local haematomatas developed at the site of injection all disappearing within a few days in spite of continued injection treatment

A few patients experienced local pain after the injections With sharp, fine gauged needles and slow and carefully superficial subcutaneous injections this disadvantage could be eliminated One patient stated that the pain was intolerable and it was necessary to discontinue injection treatment

B Laboratory results

1 Plasma lipids

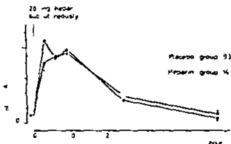
The similarity between the two groups before treatment with regard to the concentration of plasma lipids is seen in table IV The cholesterol values varied between 187—395 mg/100 ml in the heparin group and 197—436 mg/100 ml in the control group The corresponding figures for triglycerides were 0 82—4 05 m moles/l and 0 87—3 69 m moles/l The mean values for the concentration of cholesterol and triglycerides in plasma did not differ significantly between the heparin and the control group at any time during the treatment period (table IV) There occurred, furthermore, no significant change in either group in the levels of the plasma lipids during the entire study

In addition it is of considerable interest that there was no discernible effect on the fasting levels of triglycerides or cholesterol 24 or 48 hours after the injection of heparin as revealed from the samples taken on different days (table IV)

The values indicate that the subcutaneous injection of 200 mg of heparin twice a week had no acute (24 hours) or chronic (years) effect on the concentration of plasma lipids in the fasting state

2 Lipoprotein lipase activity (LLA)

The results are presented in fig 4 The LLA increased and stayed at an elevated



F. 4 Lipoprotein lipase activity in plasma after 200 mg of heparin given subcutaneously to nine patients in the placebo group and 14 in the heparin group (1) The patients had been on the treatment for several years Mean value ± standard error of the mean

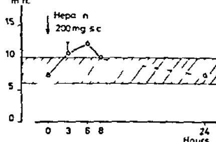


Fig 5 Coagulation time after 200 mg of heparin subcutaneously Mean value ± standard error of the mean for 12 patients The hatched area gives the normal range

level between 3 and 10 hours after the injection of heparin This level 0 05 μmoles FFA/ml/min is however not too impressive It is about 1 4 to 1/5 of the maximal LLA in plasma seen after the intravenous injection of 0 1 mg kg body weight (2) The amount of activity present in the plasma after 24 hours is slightly higher than and after 48 hours similar to that before the injection

The similarity of the values in the two groups indicates that there was no difference in the response of LLA between the two groups The heparin treatment had evidently not caused any prolonged increase of the LLA or any exhaustion in comparison to placebo treatment

3 Coagulation time was slightly prolonged after the heparin injection fig 5 The prolongation which was demonstrable after 3 hours had almost disappeared 5 hours later After 24 hours the coagulation time was the same as before the injection

Discussion

It is well known that many factors such as age sex hypertension diabetes mellitus plasma lipid levels and a variety of environmental factors are intimately associated with the occurrence of ischaemic heart disease To avoid getting a too heterogeneous group of patients we chose to study only men below 65 years of age without hypertension and diabetes No selection was made with reference to initial lipid levels As is evident from tables II and IV and fig 1 the two groups were similar in composition with regard to different variables as a result of this selection and the previously described stratification and random allocation

The difficulties involved in evaluating the clinical results of a study of this type are obvious Our initial selection criteria for ischaemic heart disease were the existence of a previous myocardial infarction and or angina pectoris We have however chosen to use only the occurrence of myocardial infarctions as

cases We have now demonstrated that when a group of patients with moderately elevated mean triglyceride level and containing no so called essential hyperlipaemia (highest triglyceride concentration 4 m moles/l) is given 200 mg of heparin twice a week subcutaneously there is no effect on the fasting values for cholesterol and triglycerides in plasma The therapeutic implications of this are obvious From the metabolic point of view it is conceivable that heparin only releases lipoprotein lipase temporarily into the blood stream without causing any net increase in total amount of lipoprotein lipase available for removal of triglyceride rich lipoproteins from the blood The triglyceride metabolism might thus not be changed when the lipoprotein lipase does not circulate in the blood The only occasion when the triglyceride metabolism might be affected would be when significant amounts of lipoprotein lipase are present in blood With our treatment schedule that would be for about 12 hours after the injection

Summary

Ninety one male patients with ischaemic heart disease but below 65 years and without complicating diseases were randomly distributed into two groups Both were, in a double blind study treated by subcutaneous injections given by a special nurse twice a week for up to five years The injections contained 200 mg of heparin for one group and a placebo for the other

After three years one or more incidents (definite myocardial infarction or sudden death) had occurred in seven patients in the heparin group, and in 14 patients in the placebo At the end of the treatment the corresponding figures were eight patients in the heparin group and 16 in the placebo The statistical as well as the clinical significance of these findings are discussed

The subjective improvement was evaluated by yearly control examinations About 75 % of the patients in both groups reported definite improvement at all these examinations

The concentration of cholesterol and triglycerides in fasting plasma was followed yearly No significant changes occurred in these values over the five years In all patients in both groups we also determined yearly the lipid levels 24 and 48 hours after the injections Followed in this way the injections had no significant acute effects on the plasma lipids

Lipoprotein lipase activity in plasma in response to the heparin injection was determined after a few years of treatment in patients from both groups and no difference in response was observed The activity increased and stayed at a level between three and ten hours after injection At 24 hours the activity was slightly above the pre injection level

The coagulation time responded with a slight prolongation, persisting for about eight hours, to the heparin treatment used

Acknowledgement

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From the Department of Anaesthesia (Head Jon Gjessing M D) Central Hospital Sundsvall Sweden

Pleural Dialysis

By

Jon Gjessing

In 1918 Ganter (1) who was the first to use peritoneal dialysis in man made experiments with pleural dialysis and found the clinical effect to be good About 40 years later Lindholm (4) described a case of acute renal failure which he treated with pleural dialysis because of the risk of hemorrhage with the artificial kidney, and where a paralytic ileus prevented the use of peritoneal dialysis He used two catheters in the pleural cavity, one for infusion and the other for drainage with suction The quantity of dialysate fluid remaining in the pleural cavity never exceeded 200 ml His conclusion was that the pleural dialysis is a clinically serviceable method when the artificial kidney or peritoneal dialysis cannot be employed

Shumway (6) in 1959 found that pleural dialysis was a relatively efficient method for removing non protein nitrogenous waste products from the blood in uremic dogs In 1963 Mandelbraun and Schumacker (5) reported from experimental studies on dogs with pleural and peritoneal dialysis that the peritoneal type was more effective and that the difference was likely to be explained on the basis of the greater surface area of the peritoneum as compared with the pleura

Material and methods

Two patients have been treated with pleural dialysis One who had previously been dialysed peritoneally 12 times because of therapy resistant edema and in whom no free peritoneal cavity could be found The other patient had renal shut down after operation for a bleeding ulcer he had chronic pyelonephritis and only one kidney At first the latter was dialysed peritoneally but this was unsuccessful because of leakage from the operation wound

Samples were taken both from the blood and the dialysate fluid and examined for creatinine protein and glucose and the vital capacity was measured during the procedure

Under local anesthesia a Bulow catheter was inserted percutaneously by a trochar in the right pleural space on the anterior axillary line 4th intercostal space After the catheter

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From Medical Department B (Head A Tybjærg Hansen) Rigshospitalet Copenhagen and The University Institute for Experimental Medicine (Head Jens Bing), Copenhagen Denmark

Simultaneous Determination of Renin Activity and Angiotensin Concentration Levels in Human Plasma

By

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The aim of the present study is to investigate the effect of mild as well as severe sodium depletion on plasma renin activity and angiotensin concentration levels simultaneously determined

Methods

The procedure used for measuring

I *Angiotensin concentration* in plasma is a modification of Boucher s method (2) Deviations from the original method are as follows

1 50—100 ml of arterial blood is collected into a Fenwal blood pack immersed in icewater Eight ml of citrate/glucose is used as anticoagulant The collection of blood using the arterial pressure as driving force takes 3—5 min within this period the temperature in the blood pack does not exceed 8° C Loss of angiotensin due to absorption by tubing and blood pack cannot be demonstrated

2 Analytic grade of resin (Bio-Rad AGw 50x2) is employed to eliminate the risk of an obstructed plasma flow through the column

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3 In order to eliminate the initial depressor effect often seen when the extract is injected into the rat a butanol extraction is performed The eluate of the resin column is evaporated until a volume of 2—3 ml is left Temperatures from 10—50° C The residue is transferred to a siliconized centrifuge tube The flask is washed four times with 0 5 ml of 0 1N acetic acid which is also transferred to the tube The content of this tube is extracted twice with 5 ml n butanol The flocculate formed is centrifuged before each pipetting of the butanol into a round bottomed flask The butanol extract is evaporated to dryness washed twice with 80 % ethanol which is evaporated also The dry residue is now deposited on the starting line of the chromatography paper

4 The solvent front is allowed to migrate for 17 hrs (30 cm) After drying the paper two strips 2 cm wide are cut parallel to the starting line at a distance of 2—6 cm This has been technically easier and it has been proved that the angiotensin is always recovered from this small area

5 The end product is dissolved in 0 5 ml of a mixture containing physiological saline and 20 % ethanol 1 1

Fifteen recovery experiments have been carried out 5—10 ng of synthetic angiotensin were added to 15 ml plasma obtained from stored blood (1—21 days old) the angiotensin of which was previously checked

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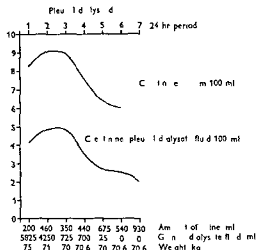


Fig 1 The creatinine concentration in blood and dialysate fluid the dehydrating effect weight loss and urinary output during seven days of pleural dialysis

had been fixed, one l of peritoneal dialysate fluid was infused and stayed there for 30 min , after which the dialysate fluid was drained into a bottle standing on the floor When the pleural cavity was empty a new infusion was started With no complications in the dialysis 20—30 dialysing baths could be introduced over a period of 24 hours

Results and discussion

A Influence of pleural dialysis on ventilation

The vital capacity was tested with the patient sitting in bed before and after the instillation of the dialysate fluid With the dialysate fluid in the pleural cavity the vital capacity was reduced 10—15 % The author (2) has found the same reduction of the vital capacity when 2 l of dialysate fluid was installed into the peritoneal cavity

B Creatinine clearance

To establish the efficiency of the pleural cavity as a dialysing membrane compared to the peritoneal cavity the clearance of creatinine has been used

The clearance has been defined as

$$C_X = \frac{(X)_D\ V_D}{(X)_B\ T_D} \quad \text{where } C = \text{clearance},$$

$(X)_D$ = concentration of X in dialysate fluid $(X)_B$ = concentration of X in blood, V_D = volume of dialysate fluid, and T_D = time in minutes for the dialysate fluid to remain in the pleural or the peritoneal cavity The concentration of the substance (X) in the dialysate fluid after 30 min over its concentration in the blood has been called the concentration ratio (CR)

An average value of the concentration ratio and the clearance of creatinine was *obtained from one of the patients on several* occasions during both pleural and peritoneal dialysis from the other patient values were obtained only during pleural dialysis

With 1 l dialysate fluid remaining in the pleural cavity for 30 min , the clearance of creatinine and CR were 17 74 ml/min and 0 47 The peritoneal clearance and CR under the same conditions were 18 47 ml/min and 0 46

When the amount of dialysate fluid was increased from 1 to 2 l per 30 min in the peritoneal cavity, the clearance increased to 34 5 ml/min Tenckhoff et al (7) found the same increase in clearance when the flow rate increased

C Protein loss and glucose absorption

Almost the same amount of protein was lost as in peritoneal dialysis with an average value of 0 2 g%

The absorption of glucose from the dialysate fluid, containing 1 5 % glucose, was less (5—6 g/l) The dehydration effect with 7 % glucose in the dialysate fluid was almost the same in pleural as in peritoneal dialysis (3)

Summary

Pleural dialysis is easy to perform, but somewhat more painful than peritoneal dialysis The efficiency seems to be almost as good as in peritoneal dialysis, with the vital capacity only slightly decreased

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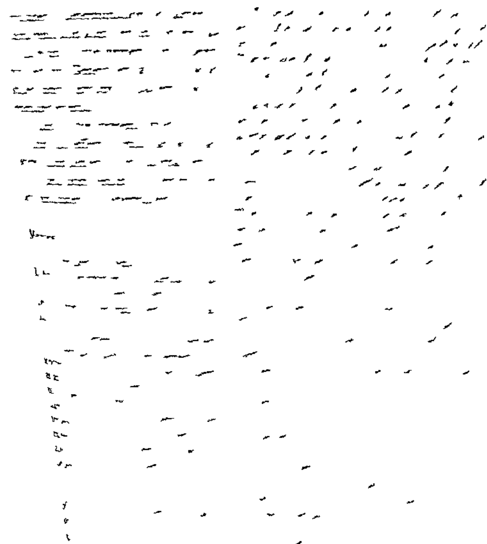
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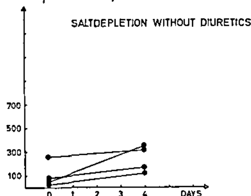
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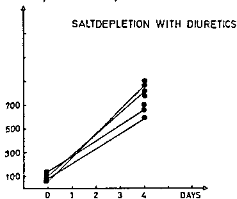
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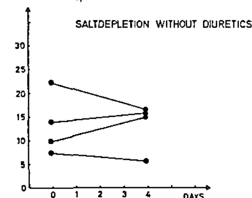
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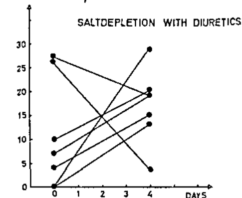


Fig 1 Renin activity levels and angiotensin concentration levels before and after sodium depletion with/without natriuretic agents Due to the lack of plasma 3 determinations of renin activity before sodium depletion + diuretics were not carried out

and found to be zero The recovery percentage in these experiments was in mean 74 % ± 10 4 SD (range 58—98)

The mean value in 15 normal subjects was found to be 13 ng/100 ml plasma ± 8 7 ng SD In two cases angiotensin was undetectable

II The procedure used for measurement of *plasma renin activity* is that of Boucher s (2) with the modifications published by the author in 1965 (6) The collection of blood has been carried out as described above The treatment of plasma, incubation and angiotensin extraction has been done according to the principles of Boucher, though plasma incubation prior to salt depletion has been carried out for a 4 hour period The end product is dissolved as described earlier Eleven analyses were done in normal subjects The mean value was 112 ng angiotensin/100 ml plasma/3h ± 68 ng (SD) The coefficient of variance determined in ten analyses in duplicate was found to be 11 8 %

III *Bio assays* in rats were done as follows 0 1—0 2 ml of the 0 5 ml end product is

injected intravenously into barbiturate pentolonium treated rats with direct measurement of the blood pressure in the carotid artery The unknown solution is injected twice alternating with injections of a standard angiotensin solution containing 40 ng/ml in doses that give higher and lower pressor responses compared to the unknown solution The rise in blood pressure is directly proportional to the logarithm of angiotensin dose This linear relationship enables us to determine the amount of angiotensin in the test solution by interpolation (10) Only rats responding to 1 ng of angiotensin and insensitive to injections of 0 3 ml physiological saline are used The pressor material from human plasma shows the following similarities with synthetic angiotensin identical pressor response curve in the rat assay, identical migration in two different paper chromatographic systems

a) n butanol—water—acetic acid 45 50 10
b) isopropanol—sec butanol—water—phosphate buffer pH 8 7 7 5 2

Material

Fifteen subjects two females and 13 males from 15—63 years of age who had never shown symptoms of hypertension congestive heart failure and kidney disease None of the patients received drugs Eleven subjects were maintained on a dietary sodium intake restricted to 10 mEq/day for four days Prior to this diet their sodium intake was unrestricted Seven of the 11 subjects received a natriuretic agent (chlorthalidone 100 mg) on the fourth day of the diet Plasma-electrolytes were determined immediately prior to the onset of the dietary restriction Urinary sodium and plasma sodium were measured daily during the experimental period

Blood samples for determination of angiotensin concentration and renin activity were withdrawn immediately before the onset of the die and after four days of dietary sodium restriction The blood was withdrawn between 8—9 a m. with the subjects fasting and recumbent for eight hours prior to blood collection

Results

Before sodium depletion

Renin activity in 11 subjects Mean 112 ng/100 ml plasma/3hrs ± 68 ng (SD) Range 45—263 ng

Angiotensin concentration levels in arterial plasma in 15 subjects Mean 13 ng/100 ml plasma ± 8 7 ng (SD Range 0—26 ng In two subjects angiotensin was undetectable (fig 1)

After dietary sodium depletion without administration of natriuretic agents

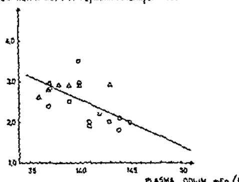
Renin activity in four subjects Mean 227 ng/100 ml plasma ± 93 ng (SD Range 112—333 ng The mean i significantly increased compared with the normal mean (0 025 < p < 0 05 t = 2 3, n = 13)

Angiotensin concentration levels in four subjects Mean 12 ng/100 ml plasma ± 3 6 ng Range 6—16 ng The mean is not significantly different from the normal mean (0 80 < p < 0 90 t = 0 16, n = 20) (fig 1) In all instances except one (the case with the greatest decrease in angiotensin concentration see fig 1) the difference between the two measurements is within the 95 % confidence limits of the accuracy of the method

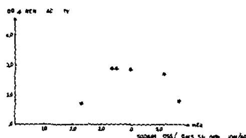
After dietary sodium depletion with administration of natriuretic agents

Renin activity in seven subjects Mean 770 ng/100 ml plasma/3 hrs ± 101 ng (SD) Range 600—889 ng The mean is significantly different from the normal mean (p < 0 001, t = 17 2 n = 16)

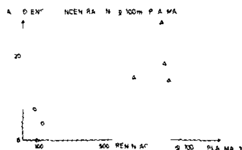
Angiotensin concentration levels in seven subjects Mean 17 ng/100 ml plasma ± 6 7 ng (SD) Range 4—28 ng



F g 2 Correla on between ren n act v ty log ren n act v ty) and plasma sod um Before salt deplet on After salt deplet on □ —d uret cs △ + d uret cs



F g Correlat on bet een log ren n act v ty and s d um loss 4 days of sod um restr ct on kg Salt d pl on d uret cs △ salt deplet on d r cs □



F g 4 Correlat on bet een ang otens n con centra on levels and ren n act ty Bef re salt deplet on after salt deplet on d uretics + d uretics

The mean is not significantly different from the normal mean 0 10 < p 0 20 t = 1 4 n = 23) fig 1 In all instances the difference between the two measurements exceeds the 95 % con fidence limits of the accuracy of the method The two cases of sharp decrease of angiotensin concentration must be noted The reason for this remains obscure

Correlation between renin activity (log renin activity) and plasma sodium

Corresponding values of plasma sodium and log renin acitivity are plotted in a coordinate system Values from normal subjects as well as sodium depleted subjects (with/without diuretics) are included Twenty pairs of observations A linear correlation is demonstrated with r = −0 74 (significant for p < 0 001 t = 4 5 n = 18) (fig 2)

Correlation between renin activity and sodium loss/4 days of sodium restriction/kg

Log renin activity and sodium loss *per four days of sodium restriction per* kg are plotted in a coordinate system (fig 3) Ten pairs of ob ervations are used The scatter plot shows no correla tion

Correlation between renin activity and angiotensin concentration levels

As well normal values as values aft r dietary sodium depletion with/without diureti s are used Eighteen sets of observations are used The scatter plot demonstrates no correlation (fig 4)

Correlation between angiotensin concentration le els and plasma sodium

Normal values as well as values after dietary sodium depletion with/without diuretics are plotted Twenty pairs of

observations are used The scatter plot shows no correlation (fig 5)

Correlation between angiotensin concentration levels and sodium loss/4 days of sodium restriction/kg

Angiotensin concentration levels and sodium loss per four days of sodium depletion per kg are plotted Ten sets of observations are used No certain correlation is demonstrated (fig 6)

Discussion

Our observations as to normal values of renin activity and angiotensin concentration correspond to the values stated in the literature, as regards renin by Boucher et al (2) and Cohen et al (5) using the same method as regards angiotensin by Massani et al (7) who apply a closely related method

The increase in plasma renin activity in subjects on sodium restriction with and without natriuretic agents generally agrees to values found in similar experiments performed by Genest et al (6) Brown et al (3)

The inverse linear relationship between renin and plasma sodium shown by us in normal subjects is also stated by Brown et al (4) in hypertensive patients irrespective of etiology complications treatment or B P at the time of sampling (39 sets of observations r −0 472 $p < 0.001$) In contrast Meyer et al (8) are not able to establish such a correlation in a similar group of hypertensives

Remarkably there is no relationship between renin and sodium deficit The difference between the means of sodium loss in the group with and without

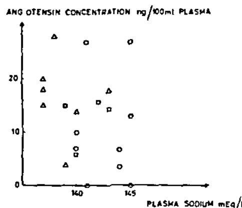


Fig 5 Correlation between angiotensin concentration levels and plasma sodium Before salt depletion ○ after salt depletion □ — diuretics △ + diuretics

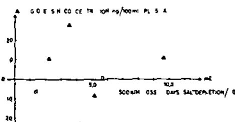


Fig 6 Correlation between angiotensin concentration levels and sodium loss 4 days of sodium restriction kg Salt depletion di uretics △ salt depletion — diuretics

diuretics (fig 3) is not significant ($p = 0.25$ $t = 1.2$) The difference between plasma renin in the same group is highly significant ($p < 0.001$ t 9 7)

Mulrow et al (9) and Barbour et al 1) have studied the angiotensin concentration in arterial plasma and renal venous blood respectively, prior to and after sodium depletion of human subjects Mulrow et al investigated healthy individuals Barbour et al patients with essential hypertension Neither of these investigators fo

significant rise in angiotensin levels Natriuretic agents were not administered Scornik and Paladini (11), in dogs submitted to severe sodium depletion, report a significant increase of arterial angiotensin level It is quite conceivable that a more pronounced depletion than the one performed in our experiments might result in a significant rise of angiotensin concentration in humans

As mentioned in the beginning of this paper Genest et al (6) have simultaneously determined renin activity and arterial angiotensin levels in various groups of patients with generalized edema, no attempt was made to correlate the paired sets of values, but in a considerable number of instances discrepancies occurred between renin activity and angiotensin concentration In the present study there is no relationship between renin activity and angiotensin concentration (fig 4 nor is there a correlation between angiotensin concentration and sodium deficit (fig 6) and plasma sodium concentration (fig 5) This agrees with Scornik and Paladini (11) but these investigators were able to establish a relationship between angiotensin concentration and Na^{24}-space ($r = -0.74$ $p < 0.01$)

Our present *knowledge* is not sufficient to explain these findings which in particular emphasize our lack of knowledge concerning angiotensin metabolism Conceivably a future measurement of angiotensin generation rate will throw light on these problems

Summary

Simultaneous determination of renin activity and angiotensin concentration levels was performed in normal subjects before and after sodium depletion with/without diuretics While renin activity increases significantly there is no significant increase in angiotensin concentration levels as to the mean values A significant correlation is demonstrated between renin and plasma sodium, but no significant relationship is found between plasma sodium and the angiotensin concentration, nor is a relationship established between increase in renin activity and total sodium loss, the same refers to angiotensin concentration levels No correlation is demonstrated between increase in renin activity and angiotensin concentration

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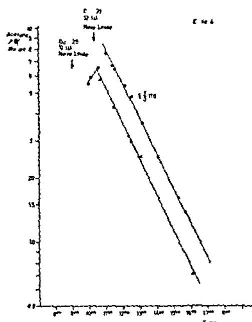


Fig 1 Case 4 A 20 year old female with 3 week history of polyuria Her first injection of insulin on October 21 Blood glucose at 7 and 12 a m and 3 and 7 p m 209 188 132 and 141 mg % On the 25th the corresponding glucose values were 132 60 73 and 174 mg %
The star indicates insulin reaction

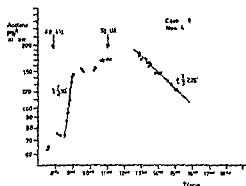


Fig 2 Case 6 A 43 year old female with a history of brittle diabetes for 16 years The patient was developing a diabetic pre-coma Her peak acetone level was 200 times the normal value Her blood glucose at 7 a m was 386 at noon 246 and at 7 p m 68 mg % The very slow disappearance rate was probably due to the pronounced ketosis

the insulin action These curves should also become valuable in testing different insulins and in regulating diabetics

Acknowledgements

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Oberex, a New Appetite-reducing Agent

By

PER BJURULF, SVEN CARLSTROM and GUNNE RORSMAN

In the treatment of obesity the most important measure is the reduction of the caloric intake of the patient Towards this end it can sometimes be helpful to give the patient in addition to a dietary regimen an appetite reducing agent There have been a number of such drugs on the market for several years Many of these have in addition to the appetite reducing action an excitatory effect on the central nervous system which must be regarded as a considerable disadvantage

We have now tested a new appetite reducing drug Oberex (Draco), which contains the substance chloforex This substance differs from chlorfentermin in having a carbethoxy substitutent on the amino nitrogen Each tablet contains 50 mg chloforex

The substance has been the subject of clinical studies in regard to the weight reducing effect (1 2 3 4) All investigators have found that the preparation has a distinct appetite reducing effect

The present aim has been to determine what further reduction in weight can be brought about with this new anorexigenic preparation employed along with the usual dietary restrictions The investigation was therefore carried out by the double blind test method with a placebo and cross over in two 14 day periods In addition 16 patients have been placed on the active preparation for six more weeks and 29 other patients for four weeks, so as to determine the tolerability of the preparation in prolonged therapy

Material and methods

The first series of patients consisted of 54 cases chosen individuals who had come to the Medical Clinic of Lund Hospital usually on the recommendation of another doctor Some patients had sought treatment for their obesity or sequelae thereof, but the series includes also patients with other internal diseases such as hypertension and maturity onset diabetes. Four cases of bronchial asthma are found in the series One individual has been excluded because he did not appear at the agreed check up This was

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later shown to be due to gallstone trouble which led to his admission to the Surgical Clinic

Hypertension defined as diastolic blood pressure exceeding 100 mm Hg and/or systolic blood pressure exceeding 200 mm Hg occurred in 20 cases Nine of these received no therapy for hypertension Diabetes mellitus occurred in ten cases, of which four were treated by dietary measures only In six cases there was treatment with oral antidiabetic drugs Medication with psychopharmaceutics of amitriptylin type was given in four cases The four cases with bronchial asthma were treated with ephedrine containing tablets and cough medicine (usually a mixture containing ammonium chloride) three also received steroids

In all cases where other therapy was given during the experimental period the dosage of the preparation was kept constant, but in the asthma cases a certain variation of the isoprenalin consumption in spray has occurred

The cases in the double blind investigation were heterogeneous in age (17—74 years, mean age 56 years) The series consisted of 11 men and 43 women Thirty three of the patients had been treated earlier for one or more periods with anorexigenic preparations

During the double blind study 100 mg Oberex were given in one dose in the morning For the prolonged therapy with the preparation 29 consecutive patients were later chosen, who were placed on 100 mg Oberex daily for four weeks Sixteen other consecutive patients (age 26—29 years mean age 52 years four men 12 women) were given 100 mg Oberex in the morning for 14 days and then 50 mg Oberex every morning for a period of one month

The patients were informed that they were going to participate in the testing of a new drug for weight reduction They were asked especially to note if they observed anything unusual or divergent during the experimental period The usual medical history was recorded along with notes on heredity in relation to obesity Regarding the obesity history the patients were asked about earlier variations in weight and whether they thought that there was a special cause of their obesity A detailed diet history was taken and then a routine medical examination performed The patients were given dietary instructions which merely converged certain general principles such as that lean meat fish and vegetables were unrestricted Only one small potato and a thin slice of bread per day were allowed Fat meat, fat fish and gravy were to be avoided completely as well as sweets and cakes Skim milk was recommended as a beverage No caloric tables or diet lists were distributed in connection with the examination The patient then received a package with 30 tablets which was marked with a letter and a number according to a special code Neither the doctor nor the patient knew whether it was the pharmacologically active or inactive preparation that the patient received Blood tests were carried out, comprising ESR, Hb leucocytes differential count, GOT GPT, creatinine/s thrombocytes and urine analysis for albumin and glucose In addition serum haptoglobin was determined in 15 cases

The participating patients were checked after 14 days when they reported any subjective trouble or discomfort A new medical examination was performed and tests were carried out according to the same pattern as earlier They received a new package of tablets with the same number but with a new letter Again the code was unknown to the doctor as well as to the patient After another 14 days the double blind examination was terminated with a new medical examination and testing

In the statistical treatment of the weight observations the weight difference between the initial value and the value after 14 days therapy was computed Those who had been treated with the active preparation during this period were then compared with the placebo group Similarly those who had been treated with the active preparation during the second half of the experimental period were compared with those who had received the placebo preparation during this period

TABLE I Shift in average weight during periods with Oberex in relation to placebo period

Weight	Pharmacologically active preparation		Pharmacologically inactive preparation		
	1st preparation	2nd preparation	1st preparation	2nd preparation	t_{diff}
M±S.E.M.	−2.26±2.02	−1.52±1.86	−1.04±1.48	−0.63±1.44	t_{diff_1} 2.524** t_{diff_2} 1.980*
t_{diff_0}	5.93***	4.17***	3.58**	2.31*	

t_{diff_0} refers to changes in relation to the initial value
t_{diff_1} refers to comparison between weight changes during periods when Oberex and placebo respectively were given as first preparation
t_{diff_2} refers to comparison between weight changes during periods when Oberex and placebo respectively were given as second preparation

In treatment of the other data the differences between the value before and that after treatment with the active preparation were computed. These were then compared partly in relation to an assumed 0-hypothesis and partly with the differences that had arisen during the period of placebo treatment. Thereby it was possible to judge whether a shift in weight and in laboratory data indicating organic damage had occurred during treatment with the active preparation in comparison with the initial value and in relation to treatment with the inactive preparation. The limits of significance are defined in the customary manner where p-values at the 0.05 level are marked with one asterisk, at the 0.01 level with two asterisks and at the 0.001 level with three asterisks.

Results and discussion

Weight reduction and subjective evaluation

When the pharmacologically active preparation was administered as the first preparation, the mean (average) weight reduction was 2.26 kg compared with 1.04 kg when the placebo preparation was given first. When the active prepara-

TABLE II Comparison between the losses in weight of individual patients during period with placebo and with Oberex. In χ^2 analysis consecutive groups have been placed together two by two. In addition Yates correction has been employed (x^2=11.9**)

	Kg	Oberex		Placebo	
Weight increase	> 2	2		2	
	1	2	4	5	7
Weight almost unchanged	0±0.9	10		18	
Weight decrease	1	8		13	
	2	12	20	12	25
	3	12		2	
	4	6	18	2	4
	5	1			
	6	1	2		

TABLE III Comparison betv een Oberex and placebo in regard to reported effect of bl nd preparat on when the pat ent v as asked to compare effect of the administered prepara tion with that of a reduc ng med c ne used previousl)

	Oberex	Placebo
Better	15	5
Equall) good	1	7
Poorer	1	3

TABLE IV Reported effect of bl nd preparat on dur ng second period in comparison wi h effect of bl nd preparat on during f rst per od for Oberex and for placebo

	Oberex	Placebo
Better	11	0
Equall) good	8	11
Poorer	7	17

TABLE V Reported s de-effects Those spon taneousl) reported are shown within parentheses

	Oberex	Placebo
Increased sweat ng	4 (?	1 1)
Dryness of the mouth or increased th rst	9 (5	5 (?)
Increased urinary output		1
Const pat on	6 (1	4 ?
Sleep ness or tiredness	6 (5)	2 (2)
Nausea	1	5 (2
D zz ness or orthostatism	5 3	4 1
Increased hunger	1	3 2
Increased energy	4 (3	5 (4
Decreased effect during 2 week period	3	0
Loss of hair	1 1	
Headache	5 (5	1
Itch ng		2 (2
Diarrhea	3 (3	

tion was administered after the period with the placebo preparation the average weight reduction was 1.52 kg compared with 0 63 kg during the corresponding placebo period The data are collated in table I This indicates that the tested preparation has a specific weight reducing effect over and above the placebo effect From table II it is seen that this effect is not limited to isolated cases only Here the material is stratified in relation to how much the individual patients fell in weight during the two periods There was also a continued weight reduction when the active prepa ration was subsequently tested for longer periods (tables VII and VIII) It was also shown that in the subjective evalua tion of the effect of the preparation there was a difference between the active preparation and the placebo The individuals have given to a significantly greater extent a positive evaluation of the effect experienced with the active preparation compared with the placebo and with other appetite reducing medi cines previously used (tables III and IV X^2 = 23 1)

Subjective side effects

In the check up examinations the doctor asked particularly about side-effects and spontaneously reported symptoms were also recorded (table V) However no significant difference could be demon strated in these respects between the active and placebo periods There has been no evidence of any excitatory effect on the central nervous system

Laboratory data

Statistical treatment of the laboratory data is collated in tables VI VII and

TABLE VI Shift in the average laboratory data during Oberex periods in relation to preceding and following placebo periods

	Pharmacologically active preparation	Pharmacologically inactive preparation		t_{diff}
		1st preparation	2nd preparation	
ESR	−1 29±8 50	0 75±8 69	3 78±5 96	t_{diff_1} 0 964 t_{diff_2} 2 757**
t_{diff}	1 086	0 423	3 291**	
Hb	0 13±0 79	0 20±1 00	−0 20±0 56	t_{diff_1} 0 314 t_{diff_2} 1 961
t_{diff}	1 197	0 981	1 881	
Erythrocytes	0 09±0 36	−0 05±0 26	0 01±0 28	t_{diff_1} 1 614 t_{diff_2} 0 812
t_{diff}	1 446	0 825	0 090	
Leucocytes	290±1 541	−536±1 493	100±1 503	t_{diff_1} 2 219* t_{diff_2} 0 516
t_{diff}	1 345	1 795	0 339	
Thrombocytes	13 520±49 849	6 000±45 643	3 558±50 051	t_{diff_1} 0 601 t_{diff_2} 0 756
t_{diff}	1 879	0 617	0 361	
Neutrophils	0 74±11 93	−2 73±10 18	−1 76±8 09	t_{diff_1} 1 234 t_{diff_2} 1 006
t_{diff}	0 449	1 313	1 171	
Eosinophils	−0 67±2 84	0 20±2 60	0 04±3 25	t_{diff_1} 0 699 t_{diff_2} 1 011
t_{diff}	1 717	0 385	0 058	
Monocytes	−0 18±5 41	−0 12±3 13	1 27±3 67	t_{diff_1} 0 051 t_{diff_2} 0 955
t_{diff}	0 241	0 192	1 830	
Lymphocytes	−0 42±10 53	2 90±7 96	1 75±8 82	t_{diff_1} 1 935 t_{diff_2} 0 917
t_{diff}	0 287	1 820	1 050	
GOT	−3 42±14 73	−0 82±13 02	−1 35±16 05	t_{diff_1} 0 710 t_{diff_2} 0 560
t_{diff}	1 607	0 295	0 135	
GPT	−0 06±22 15	1 86±17 54	1 42±29 76	t_{diff_1} 0 377 t_{diff_2} 0 244
t_{diff}	0 0195	0 498	0 244	
LDH	−3 10±49 0	27 43±52 6	−11 33±46 5	t_{diff_1} 1 870 t_{diff} 0 537
t_{diff}	0 341	1 951	0 944	
Creatinine s	0 05±0 14	−0 01±0 14	−0 05±0 10	t_{diff} 1 732 t_{diff_2} 3 015**
t_{diff}	2 305*	0 514	2 355*	

(cont)

Summary

A new appetite reducing drug, Oberex (Draco), has been tested with double blind technique and crossing-over This drug clearly has a weight reducing effect over and above the placebo effect In the subjective evaluation the patients also reported to a noticeably greater extent a better effect from the tablets containing the active preparation than from the placebo tablets The possibility that the preparation may eventually excite the central nervous system has not been objectively tested here, but questions which were directed to the patients with the idea of estimating the occurrence of central stimulation gave no evidence of such an effect One advantage of the preparation is its relatively prolonged effect which makes one dose in the morning apparently sufficient

No subjective side-effects of a serious nature have occurred In no case was it necessary to discontinue the treatment

The comprehensive laboratory examination carried out on the patients has not revealed any evidence of organic damage As mentioned in the Results, however, there is probably a hemoconcentration during treatment with the preparation In summary it can thus be said that the preparation constitutes a valuable addition to the arsenal of appetite reducing drugs previously available

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Hemochromatosis and Red Wine

By

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The most characteristic feature of idiopathic hemochromatosis is an excess of iron distributed in the parenchymal cells of the liver, pancreas, heart and other organs The origin of the disease is a changed metabolism of iron, but there is agreement neither on its cause or nature nor on the site of the disorder

Sheldon in 1927 (15) suggested idiopathic hemocromatosis to be of genetic origin, because he could advance significant objections to all the prevalent concepts He wrote, however, in 1935 (16) At that time I did not know of the existence of family cases of the disease Since then many family histories have been published even cases of hemochromatosis in twins At present the genetic theory is dominant but the pattern of inheritance is not clear

MacDonald et al (8 9, 11) argue that hemochromatosis is an acquired condition brought about by the coexistence of two factors one of excess of iron and a second of nutritional especially folic acid deficiency often associated with the development of cirrhosis The cause of the cirrhosis with or without iron is not understood, but appears not to be iron itself MacDonald and Pechet (10) have succeeded in producing hemochromatosis in experiment animals with iron excess and folic acid deficiency

The iron overload in Bantu negroes in South Africa described by Boothwell and Isaacson (3) and Seffel et al (14) is due to the high iron content in their home made kaffir beer and food prepared in iron pots and causes hemosiderosis rather than hemochromatosis

Idiopathic hemochromatosis occurs frequently in the province of Brescia which is situated in Northern Italy, east of Milan In 11 months Jacchia et al (7) found 22 cases of hemochromatosis six of which were in a clinically latent form in their Department of Internal medicine the 22 constituting 0 60 % of all admissions during the period The

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consumption exceeding 2 l a day, their disease might depend on an iron over load, especially as they were over 40 years old and thus had had time to accumulate large quantities of iron Even a wine consumption of 1 l a day of 90 mg iron/l for some 20 years or more is sufficient to give rise to hemochromatosis

The present study supports the suggestion of MacDonald (8) that "idiopathic" hemochromatosis is a disease of acquired and not genetic origin, the reported familial cases of the disease being due to the similarity to the proband in dietary habits

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The Treatment of Angina Pectoris with a New Beta-receptor Blocking Agent (H 56/28)

By

PER BJORNTORP

Adrenergic beta receptor blocking agents have been used in angina pectoris by several investigators Prichard et al (10) found a good effect with pronethalol, a substance which later however, proved to cause serious side effects Propranolol has been used in several trials in angina pectoris Srivastava et al (13) found no definite positive effect at a dosage of 20 mg t i d Gillam and Prichard (8) on the other hand in a double blind trial in angina pectoris found a significant effect at high dosages This field has recently been reviewed by Epstein and Braunwald (6)

The effect on angina pectoris of a new beta receptor blocking agent H 56 28 1 (2 allylphenoxy) 3 isopropyl amino propanol (2) hydrochloride (Aptin® AB Hassle Goteborg) has now been tested in a double blind trial The structure and synthesis of this drug have recently been described (4) as well as circulatory and metabolic effects in the experimental animal (1 3) and man (2 3 7 9) It is of interest in the latter connection that H 56 28 at the same β receptor blocking dose as propranolol differed in not causing a decrease in cardiac output, possibly because of an intrinsic sympathomimetic effects of H 56,28 (7)

Material and methods

Seventeen patients attending the out-patient department with angina pectoris were selected for the trial Four of these later had to be excluded two of them because of inability to record attacks and attend for check ups one because of obstructive lung disease previously unknown and one because of a pneumonia shortly after starting the trial The remaining 13 patients consisted of two women aged 66 and 74 years and 11 men aged 39—79 years All had with effort an anginal chest pain of typical localization, immediately subsiding at rest nitroglycerin sublingually alleviated the pain In six of the patients coronary arteriography had been done showing gravely pathological lesions in all cases Six of the patients had previously had myocardial infarction but not within three months of starting the trial All patients were having at least two attacks of angina pectoris per week lasting at least two min

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TABLE I Clinical data and results

Pat no	Age	Sex	Diagnosis	Dosage (mg × 4)	No of attacks per 14 days Control	Treatment	Placebo	Nitroglycerin consumption per 14 days Treatment	Placebo	Subjective impression[1]	B P[2] (mm Hg)
1	53	♂	Ang pect Hyperchol	70	30	21	23	—	—	+1	3/—5
2	74	♀	Ang pect Hypertension	50	11	8	10	7	10	+4	31/11
3	69	♂	Ang pect Hyperchol Hypertension	100	6	2	4	3	4	+5	42/14
4	39	♂	Ang pect Hyperchol	100	19	7	7	9	9	0	0/—9
5	51	♂	Ang pect Hyperchol	100	8	4	8	2	7	+6	8/0
6	51	♂	Ang pect Hyperchol	100	11	3	3	3	2	0	—6/0
7	52	♂	Ang pect	100	32	10	11	—	—	+1	6/5
8	66	♀	Ang pect Stenosis of carotid arteries	100	15	17	18	—	—	+6	17/7
9	59	♂	Ang pect	100	10	0	0	0	0	+4	7/0
10	56	♀	Ang pect Hypertension	100	5	3	2	0	0	+1	11/6
11	79	♂	Ang pect	80	8	0	1	0	2	+3	—14/7
12	60	♂	Ang pect	100	5	0	0	0	0	0	0/5
13	68	♂	Ang pect	100	27	11	19	13	22	+5	2/5
Total					187	86	106	37	56		

[1] General condition during treatment and placebo periods as estimated by the patient, compared with the preceding week unchanged 0 improved +1 much improved +2 worse —1 much worse —2 In the table the score of the treatment period minus the score of the corresponding placebo period is given

[2] The figures give the change in systolic/diastolic blood pressure calculated as mean systolic/diastolic blood pressure during placebo periods minus that during treatment periods

when the trial started Two of the patients were on long term treatment for hypertension and two others for hyperlipemia None had signs of obstructive lung disease or a history of serious lung disease None had shown tendencies to mental depression Two patients were on digitalis with or without saluretics because of earlier heart decompensation During the trial, no changes were made in the pre trial medication The patients were recommended not to use nitroglycerin prophylactically The trial started with a control period of two weeks, followed by a run in period with increasing dosage of H 56/28 The dose was 10 mg q i d in the first week and was increased by 10 mg q i d each week up to the highest tolerable dose or to a maximum of 100 mg q i d administered in

divisible 20 mg tablets After this run in period which accordingly in most cases was ten weeks two treatment periods and two placebo periods followed each period being two weeks These were randomized in a double blind system where two similar periods never came together The patients were not told that placebo tablets were introduced during any period of the trial The placebo tablets were identical in taste and appearance with the H 56/28 tablets

The patients were seen weekly in the morning in the fasting state They were requested to record on a data sheet issued weekly the duration and severity of each angina pectoris attack as well as nitroglycerin tablet consumption Each day was divided into four registration periods The forms were collected every week by the physician in charge of the trial who also recorded whether there had been any changes in the general condition as reported by the patient himself Possible side effects were looked into by taking a history and performing a routine physical examination The remaining tablets were counted ECG test was done weekly The following laboratory tests were done fortnightly in the fasting state hemoglobin counts of white and red blood cells and thrombocytes differential white cell count serum bilirubin thymol turbidity reaction alkaline phosphatase serum glutamin oxalacetic acid transminase serum glutamin pyruvic acid transaminase plasma creatinine free fatty acids triglycerides and cholesterol The patients were also weighed on each visit Chest X ray with measurement of heart volume was performed before the trial in all patients and after the trial in eight of the patients

Statistical calculations have been performed one tailed according to Wilcoxon rank sum test (12)

Results

The results are seen in table I and fig 1 Table I shows that ten of the 13 patients took H 56/28 in a dose of 100 mg q i d The remaining three did not take this dose, for different reasons because of increasing fatigue, probably connected with hypotension (patient no 1), because the relatives thought the patient took too many tablets (patient no 2) and because the patient himself thought he took too many tablets (patient no 11) When the control period and treatment period are compared a marked reduction of the number of attacks is evident in all patients except no 8 This difference is, however, less pronounced when treatment and placebo periods are compared, because during the placebo periods the attacks in most cases did not become as frequent as in the control period After the run in period patients nos 6, 9, 10 11 and 12 had no definitely measurable angina pectoris by the criterion of at least two attacks per week In the remaining eight patients measurable angina pectoris was present at the end of the run in period and seven of them had fewer attacks during treatment than on placebo, while in one patient (no 4) the number was unchanged This difference is statistically significant ($p < 0.01$) The nitroglycerin consumption closely matched the coming and going of attacks

The difference in frequency of attacks between control and placebo periods was analysed Fig 1 demonstrates that the frequency of attacks decreased gradually with the increase in dose during the run in period The increases in frequency during placebo periods were fairly small as compared with treatment periods There was no difference between the first and the second periods of placebo and H 56/28 respectively

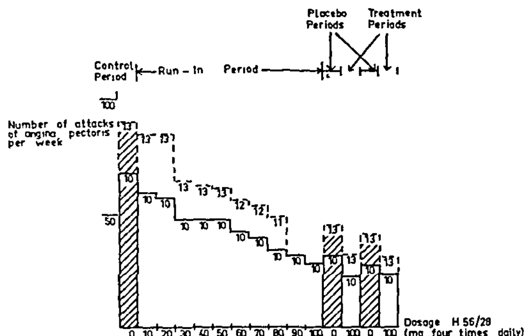


Fig 1 Number of attacks of angina pectoris during control period run in period and blind periods for the whole group of patients (dotted line), and for those ten patients who took H 56 28 at a dose of 100 mg q i d (whole line)

The numbers in the columns indicate the number of patients taking the corresponding dose of H 56 28

In order to allow of comparison of the number of attacks per week these are shown fo the blind periods also Note however that each blind period was two weeks and total treatment time thus four weeks

Table I also shows that ten of the 13 patients had a positive score when estimating their condition themselves or seven out of eight with persisting angina pectoris as judged by the frequencies of attacks In four patients a decrease of at least 10 mm Hg systolic or diastolic blood pressure was found (patients nos 2 3, 8 and 10) and in one case an increase (no 11) Three patients reported spontaneously that they felt calmer and less stressed during the treatment periods (patients nos 5, 7 and 8) Free fatty acids of plasma fell on the average by 84 μEq1 during the treatment periods ($p<0 05$) as compared with placebo periods, while plasma cholesterol and triglycerides were unchanged

Side effects

The initial blood pre sure of patient no 1 (105 85) fell to 85 60 on a rather high dose of H 56 28 The patient felt tired but neither he nor the other patients were orthostatic With reduction of the dose to 70 mg q i d the blood pressure rose and the tiredness disappeared Two patients complained of a slight feeling of pressure on the head These symptoms disappeared after two weeks Two patients complained of slight constipation

The patient later excluded because of pneumonia had increased dyspnoea in the run in period but no other signs of heart decompensation After increasing the digitalis dose this symptom disappeared No other patients have shown any signs of heart decompensation

None of the patients going through the whole trial has experienced during it any severe pain of long duration suggestive of myocardial infarction One of the patients excluded because of inability to record attacks had a period of severe pain, after which a rise in serum glutamic oxalacetic acid transaminase was noted but there were no electrocardiographic changes Patient no 10 with constantly pathological liver function tests (probably due to alcoholism) had a rise in this transaminase level from 140 to 224 during Easter holidays These values later returned to about 140

Chest X ray including heart volume was similar before and after the trial

No other pathological changes in the laboratory tests ECG or body weight have been noted One patient with a thrombocyte count of 50,000 in the control period had normal counts in the run in period

Discussion

In the patients who still had angina pectoris attacks after the run in period there were fewer attacks per week during treatment periods than during placebo periods The difference in number of attacks is very small in most cases but has statistical significance such that its clinical implications warrant discussion

Attacks during treatment periods are much less frequent than during the control period As a matter of fact five patients hardly had any angina pectoris left after the run in period For estimation of the clinical value of the treatment it is of interest to analyse the difference between the control and the placebo periods in the frequency of attacks Cole et al (5) found a decrease in frequency under circumstances similar to those of the run in period in the present work This decrease was similar whether drug or placebo was given On the average fall in frequency of attacks occurred over 11 weeks in good agreement with the present work Some patients were completely free of angina pectoris after the run in period

The establishment of a good psychological relationship between physician and patients during the run in period thus undoubtedly helped to cut the number of attacks of angina pectoris during this period

The trial took place during hard winter with no definite changes in weather or temperature It therefore seems unlikely that such factors could have contributed

Several patients said that they could manage more physical activity without chest pain during the run in period Patient no 8 initially had to stay indoors but after starting the trial she could leave her house and walk Patients nos 3 6 7 9 12 and 13 could increase their walking distances and other physical activities This increased ability to perform work may have contributed to a physical training of these patients with a lower

HERMAN ZONDEK

On the occasion of his eightieth birthday September 4, 1967

Professor Herman Zondek is one of the pioneers in clinical endocrinology. His active period in this sphere has lasted for more than *five decades and even during the later years* several articles from his hand bear witness of his deep knowledge of this field of medicine. Deep knowledge not only of endocrinology but also of adjacent areas made him one of the outstanding clinical endocrinologists of our time. It may seem unbelievable today that anybody could ever have undertaken the task of writing a text book in endocrinology by himself. When first published in Germany in 1923 Zondek's text book was one of the first in endocrinology, and one of the best. The book has kept abreast with the enormous progress in medicine and appeared in many revised and enlarged editions in all major languages. The book reflects Zondek's tremendous clinical knowledge and experience chiefly derived from his patient material. It also demonstrates the impact of Herman Zondek's life-work.

It is not easy to choose today among his prolific writings and say: This particular work confirms the ingenuity and imagination of Herman Zondek. But I would not be surprised if he himself, if given the choice would pick out his early (1918—20) description and elucidation of the changes in the heart in thyroid insufficiency, the myxedematous heart. *Not only because of its master*ful description of the patho-physiological features of this clinical entity — but even more so because these early papers and several later ones display so much imaginative thinking and critical analysis. Herman Zondek's articles from five decades provide the reader with a survey of the development of endocrinology during its most fruitful years, from the speculative era to its present period with its close relations to the basic sciences. It is interesting to note how often his thinking has followed paths which would not reach their goals until much later, not until bio-chemistry had provided information regarding the structure and mechanisms of actions of hormones.

When reading Herman Zondek's papers today one feels great satisfaction that he has been given the privilege to live long enough to see how often his ideas and predictions were right. This kind of experience is rare in an as rapidly developing sphere of medicine as endocrinology.

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A Controlled Study of the Effect of Treatment on Chronic Bronchitis

An evaluation using pulmonary function tests

By

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The etiology of chronic bronchitis is unknown However, various bacteriological and pathophysiological findings have led to advocacy of different treatments

The causative role of infection in exacerbations and in persistence of the disease is stressed by the demonstration of inflammatory changes in the bronchi (27), and by the frequent occurrence of purulent sputum with haemophilus influenzae and pneumococci (11 20 30) but often the former has been of the non encapsulated form and the latter of the higher avirulent types (30 32) A positive correlation between the presence of these bacteria the purulency of the sputum, and the clinical condition of the patients has been found by some authors (14, 22) but not by others (30)

On treatment with antibiotics directed against the bacteria observed in the sputum the effect has often been poorly correlated with changes in the clinical condition of the patients (8 13 15)

Chemotherapy in chronic bronchitis has also been evaluated by observing the number, length and severity of acute exacerbations Earlier studies entailing long term administration have been reviewed by Davis et al (11, 12), who concluded that no convincing evidence existed in favor of this treatment as was borne out by their own trial of the effect of tetracycline and chloramphenicol Later investigations from other clinics have indicated that long term administration of antibiotics (mainly tetracyclines) reduces mainly the duration rather than the number of acute exacerbations of the disease (8, 13 16 17, 18) The evaluation of these studies is difficult as the clinical response to the treatment was judged merely from the patients own statements and from an overall impression gained by different physicians and health visitors In addition the patients in some studies received other treatment including antibiotics,

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bronchodilatators, intermittent positive pressure breathing etc Moreover, the favorable results of tetracycline treatment in a commonly cited report (16) is due mainly to results from three of 16 participating clinics comprising less than 15 % of the material

A number of investigators have also evaluated long term chemotherapy by pulmonary function tests Cherniack et al (8) and Norman et al (25) observed definite clinical improvement following tetracycline but could not detect any change in pulmonary function The studies of Hallet et al (19) with erythromycin and of Davis et al (11, 12) with tetracycline or chloramphenicol revealed neither clinical improvement nor changes in pulmonary function

It has also been suggested that the factors initiating the exacerbations are not bacterial Murdoch et al (24) demonstrated a significantly raised influenza virus antibody titer in approximately 20 % of 75 bronchitics during the exacerbations, and Cavilli et al (7) found that 52 % of all exacerbations were caused by viruses or mycoplasms Fog tobacco smoke and industrial fumes have also been found important in eliciting exacerbations of chronic bronchitis (14 26 32)

Various expectorants are widely but empirically used in the treatment of chronic bronchitis particularly potassium iodide Recently it has been postulated (21) that potassium iodide might have a specific role in liquefying purulent secretions as it induces enzymatic hydrolysis of sputum protein In a clinical study however, Alstead (1) could not demonstrate any effect of potassium iodide either on the gross appearance of the sputum or on the amount secreted

Another widespread therapeutic measure in the management of chronic bronchitis is breathing exercises Campbell and Friend (6) found no effect of this kind of treatment in a carefully selected group of patients followed with pulmonary function tests and electromyography Anthonisen et al (2) did find a significant improvement in arterial oxygen saturation and carbon dioxide tension both in patients treated with various physiotherapeutic measures and in a control group Many of the patients in their study, however, also received treatment for heart failure, which is known to improve lung function (31) Finally Miller (23) has reported greatly improved alveolar ventilation as indicated by blood gas analyses, but the study unfortunately lacked a proper control group

The present study concerns the possibility that an objective evaluation mainly based upon pulmonary function tests may make it possible to detect, in a strictly controlled study, any effect of various treatments of exacerbations in chronic bronchitis The study has comprised four groups of patients one (A) receiving placebo, one (B) receiving physiotherapy especially breathing exercises one (C) receiving an expectorant, and finally one (D) receiving chemotherapy

Material and methods

The material consists of 43 patients 27 men and 16 women who were admitted to the ward for acute exacerbations in chronic bronchitis during the period from Oct 1,

1964 to May 1, 1965 The criterion used for the diagnosis of chronic bronchitis was a story of cough and expectoration on most days during at least three consecutive months in each of two or more successive years Most of the patients could be classified as having chronic mucopurulent obstructive bronchitis (3)

Only patients in the age group from 45 to 75 years were selected The mean age was 64 years Subjects with severe deformities of the spine or chest, with localised or generalised specific lung disease or with signs of cardiac insufficiency were not included in the study In two patients the ECG showed signs of right ventricular hypertrophy

Five patients who were initially studied were later excluded Two of these developed signs of cardiac insufficiency one was found to have a bronchial carcinoma one left the ward before the end of the trial and one had to be excluded because of lack of cooperation in the pulmonary function tests

General procedure

On the day of admission to the ward the patients were selected for the study by two of us (VE and CM) The following morning (day 0) pulmonary function tests were performed To secure a homogeneous distribution of light and severe cases in the different treatment groups the patients were classified according to whether their residual volume comprised less or more than 50 % of the total lung capacity The patients in each of these classes were next allocated among four groups according to a table of random numbers

A The placebo or control group

The patients received 10 ml 3 3 % potassium chloride three times daily and calcium lactate 0 5 g four times daily with no other treatment

B The physiotherapy group

The patients received daily physiotherapy under a program individually tailored in the light of previous physical examination In addition they were given potassium chloride and calcium lactate as in group A

C The expectorant group

The patients received 10 ml 3 3 % potassium iodide three times daily and calcium lactate as for groups A and B

D The chemotherapy group

The patients received chloramphenicol 0 5 g four times daily and potassium chloride as for groups A and B Group A was thus in relation to group B a proper control group while in relation to groups C and D it was a placebo group It was not feasible to give the patients in group A sham physiotherapy

On day 0 frontal and profile roentgenography of the chest was carried out All patients allocated to groups A and B were examined clinically by one of us (PH) with respect to the musculo-skeletal system posture and especially the mechanics of breathing The patients in group B received an individualized treatment according to the result of this examination The treatment consisted of instruction in how to cough properly, diaphragmatic and abdominal breathing exercises exercises to foster a correlation of the costal and abdominal breathing and a correction of existing abnormal level of respiration postural abnormalities of local dysfunction of the thoracic cage There were also exercises to train the respiratory muscles and teach the correct ed breathing movements Some patients also received postural drainage combined with tapottement squeezing and expansion training

All patients were kept in bed in the same ward to secure uniform nursing Rectal temperature was measured mornings and evenings and the 24 hour sputum volume was measured daily The gross appearance of the sputum was noted but bacteriological examinations were not made as their usefulness seems doubtful (20 30)

The ESR (Westergren) was determined on days 0 3 6 and 10 Determinations of vital capacity and of peak expiratory flow rate were repeated on day 3 and 6 On day 10 all the pulmonary function tests made on

day 0 were repeated and groups C and D were terminated

Any effect of physiotherapy could hardly be expected to occur in ten days In groups A and B therefore, the treatment was extended to 28 days and the pulmonary function tests were repeated on the last day In this latter period the patients in groups A and B were up and about in the ward Two patients in group A did not complete the 28-day period. One suddenly deteriorated and died after 24 days Autopsy revealed chronic cor pulmonale as the cause of death Another patient with a duodenal ulcer developed signs of pyloric stenosis and was withdrawn from this part of the study

The lung function tests which were performed on day 0 and 10 (and day 28 for groups A and B) included

1 Lung volumes

Vital capacity (VC) and expiratory reserve volume (ERV) were measured in a 6 l spirometer The best of three or four attempts was used Functional residual capacity (FRC) was determined in a closed oxygen filled system connected to a Nitrograph (N V Godart) A blower secured complete mixing in the system Duplicate determinations with an interval of at least 20 min were made Residual volume (RV) was given by FRC − ERV and total lung capacity (TLC) by VC + RV All lung volumes were corrected to BTPS

A change in VC of more than 15 % and a change in RV/TLC × 100 of more than 5 % were considered significant

2 Peak expiratory flow

Peak expiratory flow (PEF) was measured with a Wright peak flow meter (33) The best of five blows was used An increase in PEF of more than 15 % was considered as a sign of improvement

3 Ventilation

Tidal volume (V_T) respiratory frequency (f) and ventilation (V_E) were measured in the course of the N_2 wash-out

4 Distribution

An assessment of the distribution of respired air was made by an open-circuit multiple breath technique (10) with continuous registration of the N_2% during oxygen breathing The N_2% after 7 min was registered as the so-called mixing index (MI)

A pathological N_2 wash-out curve with a MI of more than 2.5 % was regarded as definitely indicative of uneven ventilation (5, 10) An improvement of gas mixing is said to occur when a pathological N_2 wash out curve becomes less irregular, and the MI decreases

5 Ventilation perfusion

An assessment of the presence or absence of severe disturbances in the overall ratio of ventilation to perfusion (V/Q) was attempted by measuring

a The pattern of CO_2 elimination during one expiration as seen in capnographic tracings These were obtained by continuous registration of the CO_2 percentage in respired air by a Capnograph (N V Godart) (5)

b The arterial hemoglobin oxygen saturation (HbO_2) which was determined by the hemoreflector method (Kipp) according to Zijlstra (34)

c The physiological dead space (V_D) as computed from Bohr's formula $V_D/V_T = \frac{P_aCO_2 - P_ECO_2}{P_aCO_2}$ The arterial PCO_2 (P_aCO_2) was determined by the micro method of Siggaard Andersen (29), and the PCO_2 of mixed expired air (P_ECO_2) by the method of Scholander (28) after collection in a Douglas bag

d The arterio-alveolar PCO_2 gradient (9) The alveolar PCO_2 (P_ACO_2) was estimated from the capnographic tracings

When three of these four parameters were pathological this was taken to be strongly suggestive of disturbances in the ventilation perfusion relationship An improvement was said to occur when three of the parameters improved An improvement in the respective parameters was considered to have taken

TABLE I Normal values of lung function tests Data from 28 men and 22 women over 45 years old The regression formulas give the vital capacity in ml

	Mean	SD
VC (♂) = —2830—29 4×X+47 6×Y		
VC (♀) —2890—14 2×X+39 2×Y		
RV % of total lung capacity (♂)	39	5
RV % of total lung capacity (♀)	43	4
PEF (♂) (l/min)	480	90
PEF (♀) (l/min)	350	50
MI (N_2%)	<2 0%	
$PaCO_2$ (mm Hg)	40 1	2 4
HbO_2 (% saturation)	96 1	0 7
V_D/V_T	0 35	0 05
Arterio-alveolar PCO_2 gradient	1 7	1 7

X = age in years
Y = height in cm

TABLE II Composition of the treatment groups Listed values are pre treatment data

	A	B	C	D
No	10	10	9	9
Men	6	7	6	4
Women	4	3	3	5
Mean age	61	64	66	64
Duration of disease >10 years	7	8	6	6
Mean 24 hrs sputum vol (ml)	36	35	16	25
Mucopurulent sputum	8	7	6	6
Mucoid sputum	2	3	1	2
No sputum	0	0	2	1
Temperature >38 C	5	3	3	4
Mean ESR (mm/hr)	24	23	39	14
ESR >50 (mm/hr)	2	2	4	0
PEF <240 l min (♂) or 175 l/min (♀	5	7	6	6
VC <70%	3	4	5	4
RV % >50%	5	6	6	6
Signs of uneven ventilation	4	3	3	4
Signs of V Q disturbances	2	2	4	2

A = control group
B group receiving physiotherapy
C — group treated with potassium iodide
D — group treated with chloramphenicol as the active ingredient

TABLE III Resul s of treatment g en in the d fferent groups (cf table II) The results obta ned on from day 28 w h p e treatment data is also g en The number of pat ents w th a day O For defin t ons of impro ement or aggra at on see text

	A₀ 10			A₀ 28			B₀ 10		
	Better	Un changed	Worse	Better	Un changed	Worse	Better	Un changed	Worse
Spu um vol	?		1	—	—	—	1	10	0
Temperature	3 5	3 5	—	—	—	—	1/3	2/3	1/7
ESR	0	9	1	2	5	1	0	9	1
PEF	(	4	0	6	2	0	6	4	0
VC	3	7	0	2	6	0	2	7	1
RV %	2	8	0	?	6	0	1	8	1
Gas m x ng	1	9	0	2	6	0	0	10	0
V Q	1	9	0	0	8	0	0	10	0

pla e hen the slope of the capnograph c trac ng decreased the HbO increased by more than 3 % V_D/V_T decrea ed by more thar 0 07 and the arter o-al eolar PCO grad ent decreased b more than 4 mm Hg All tests were performed n the n orn ng by one of the authors (FSP) The tests v ere al a s carefully descr bed and taught in ad ance All tests were performed h the pa en s n the sup ne pos t on except for PEF l ch as measured v h le tl e pat ents were and ng For tl e cooperat on demand n es s VC and PEF no s gn of acqu red ab l ty to perform the ests l as been observ ed

The norm al alue of the labora ory for 50 heal h subject er 45 years of age are presen ed n table I

Compos t on g oup

A deta led e r p on f l e four treat ment groups g n n able II It is seen that the group are ery s n lar h respect to sex and age as well as to dura on of d ease

The sputum volume has been tabula ed as the a erage volume in the f r t th ee da s after admis on to the ward Apparen ly the putum volume in group C is some ha l w r than n the otl er three groups The f gures ho e er should rather be read as show ng a remarkable uniform ty between the groups as the exper ence of ourselves and others (1 4) s that sputum volume sho s a con derable day to-day variation The reasons may be that some pat ents hab tually swallow the r sputum and that in sp te of hosp taliza t on conf nement to bed and careful ins ruc t on of bo h pat ents and nurses not ncon derable amounts of sputum escape collec t on and measurement Add t on of sal a and perhaps phys olog cal variat ons in the a mount secre ed by the bronch al glands may also b factors of s gnif cance The table sho s that the gross appearance of the spu t m v as nearly id ntical in the groups

The number of pat ents v th a tempera ture abo e 38° C v as not s gn f can ly d ffer ent n the four groups

The average FSR/hr v as h gher in group C than in the other groups ESR is a often u ed but n o t unspecif c parameter and no par cular relat on bet een cl n cal cond t on the result of the lung funct on tests an l FSR v as noted

Tl e numbers of pat ents v h a severe re du on in PFF and VC — less than 50 % and 0 % of the normal values for sex an l age tal le I respect el — were equal

day 10 are compared with pre treatment data and for groups A and B a comparison of the results specified change in temperature is related to the number of patients with elevated temperature on

B_{0-28}			C_{0-10}			D_{0-10}		
Better	Un changed	Worse	Better	Un changed	Worse	Better	Un changed	Worse
—	—	—	0	9	0	2	7	0
—	—	—	2/3	1/3	—	3/4	1/4	1/5
1	7	2	3	6	0	2	7	0
6	3	1	4	4	1	5	4	0
0	9	1	4	5	0	4	5	0
0	9	1	3	5	1	3	6	0
0	10	0	0	10	0	2	7	0
0	10	0	1	8	0	0	9	0

and similarly signs of uneven ventilation or ventilation perfusion disturbances were found with equal frequency in the groups

The X ray examination of the chest showed the four groups to be homogeneous with respect to the occurrence of interstitial fibrosis and localised emphysema In about half of the patients in each group no radiological abnormalities were observed

A detailed clinical comparison of the patients in groups A and B with respect to postural abnormalities and the mechanics of breathing was made by one of us (PH) In contrast to the women, most of whom were over weight half of the men were lean with poorly developed muscles Also a large proportion of the men showed scolioses and restricted mobility of the lumbar and cervical spine and shoulders whereas kyphosis and limited function of the dorsal spine was common in both men and women In most patients the diaphragmatic respiratory function was poor and particularly in the men there was asynchronism between the costal and the abdominal breathing In the men too the thoracic cage was found to be deformed and asymmetric and the chest movements were conspiciously high costal whereas the women often showed locally fixed or disturbed thoracic respiratory function These abnormalities were found with equal frequencies in groups A and B

It may be concluded that the four treatment groups are comparable with respect to the parameters relevant to the investigation

Results and discussion

The subjective statements of the patients after the period of treatment were almost uniformly favorable and essentially similar in all four groups Almost all patients had noted improvement in general well being and in ease of expectoration besides less dyspnea These statements should of course not be disregarded, but they are poor as criteria for the effect of the treatments given They are extremely difficult to grade, and undoubtedly some patients do want to please a doctor who has shown more than usual interest in their tiresome and commonplace disease

As discussed above the volume of sputum is not a sensitive indicator of the severity of chronic bronchitis A decrease in sputum volume of more than 30 %

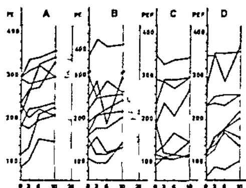


Fig 1 Variation of peak expiratory flow (PEF) in l min during the period of treatment

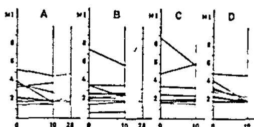


Fig 4 Variation of mixing index (MI, % nitrogen in expired air after 7 min of oxygen breathing) during the period of treatment

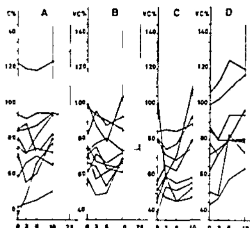


Fig 2 Variation of vital capacity (VC) as % of calculated normal value during the period of treatment

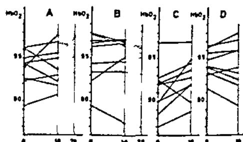


Fig 5 Variation of arterial hemoglobin oxygen saturation (HbO_2) during the period of treatment

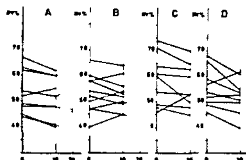


Fig 3 Variation of residual volume (RV) as % of total lung capacity during the period of treatment

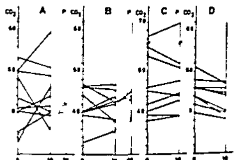


Fig 6 Variation of arterial carbon dioxide tension (P_aCO_2) in mm Hg during the period of treatment

was regarded as significant, and table III shows that such a change was observed in two patients in groups A and D, while no decrease was observed in groups B and C The table also shows the number of patients with elevated temperature who became afebrile, and the number whose ESR decreased more than 30 % It is seen that no significant differences are observed among the four groups with respect to improvement in these parameters which generally are assumed indicative of an infectious process

Among the lung function tests investigated the V_T f and V_E did not reveal any significant change nor trend with treatment in any of the groups and detailed figures are omitted from this paper

The results of all determinations of PEF, VC RV % MI HbO_2 and P_aCO_2 are presented graphically in figs 1—6 An inspection of these figures shows that the distribution of values on day 0 is similar in the four groups This strengthens the assumption that the groups are fully comparable

The overall impression given by figs 1—6 is that the pulmonary function tests which have best reflected effects of treatment are VC RV % and PEF The VC and RV % improved in several cases Concerning the latter however it must be emphasized that decreases were more often attributable to an increase in VC than to a true reduction in residual volume The number of patients showing a significant improvement in VC and RV % according to the criteria set out in Methods is given in table III Evidently an almost equal response has been obtained in the four groups, and the VC and RV % measured on day 28 in groups A and B show that no further improvement occurred during the prolonged physiotherapy

The PEF (fig 1) usually showed gradual changes when measured on days 0 3 6 and 10, but—as was the case with VC—some patients showed results markedly out of line on one of these days Such variations are usually not seen among normal subjects (31), and might indicate considerable day to-day variations in the degree of airway obstruction in patients with chronic bronchitis Approximately half of the patients in each group showed a significant increase in PEF (table III) and here also it is seen that no further improvement occurred after prolonged physiotherapy

The tests evaluating the distribution of ventilated air in the lungs and the overall ratio of ventilation to perfusion generally showed few recognizable changes upon treatment (figs 4—6) According to the criteria previously mentioned signs of improved gas mixing and of improved ventilation perfusion disturbances were found in only three and two cases, respectively after ten days of treatment and no further improvement was seen on day 28 in groups A and B (table III)

When the results of all lung function tests were evaluated for each patient a significant improvement in three or more of the parameters was found in four cases in group A and in three cases in each of groups B C and D

No further information was obtained when the response to treatment was evaluated according to sex or duration of disease

TABLE IV Clinical effects of breathing exercises in group B The number of patients with specified changes is related to the number of patients in whom a particular abnormality was found on day 0

	0—10			0—28		
	Better	Un changed	Worse	Better	Un changed	Worse
Poor diaphragmatic resp function	3/7	4/7	0	6/7	1/7	0
Asynchronous respiration	0	8/8	0	6/8	2/8	0
Asymmetric thoracic movements	0	6/6	0	5/6	1/6	0
Upper costal respiration	0	7/7	0	0	7/7	0

The effect of the physiotherapy was evaluated clinically on days 10 and 28 by the same investigator (PH) It was not possible to correct either the postural abnormalities or (table IV) the upper costal respiration, whereas the diaphragmatic respiratory function improved, in some cases already during the first ten days of treatment and in others during the following 18 days The asymmetric thoracic movements and the inappropriate timing of the costal and diaphragmatic respiratory function first showed improvement after 28 days In four patients this clinical improvement was considerable but any improvement in the lung function tests was not different from that seen in those patients who did not respond so well to physiotherapy Conversely the three patients mentioned above whose lung function showed a marked improvement did not belong among those who clinically responded to physiotherapy This discrepancy between the clinical improvement and the lung function tests is not readily explainable

A clinical evaluation of the mechanics of breathing gives only a crude view of a complicated internal process that is only partially analysed by the lung function tests used

The number of patients comprising this study is not large The data for the four groups are, however, so similar that a larger material would hardly have yielded statistically significant differences between any of the four groups

It may therefore be concluded that neither the patients own statements, nor the non specific indicators of an infective process such as sputum volume purulency temperature and ESR, nor the objective evaluation of the lung function have given any hint of a difference between groups A B, C and D in their response to treatment

This study does not point to any pulmonary function tests as helpful in choosing or rejecting patients for various forms of therapy

In view of the frequent side-effects observed during treatment with antibiotics as the penicillins and tetracyclines,

and the risk involved in uncritical use of chloramphenicol, the routine use of these drugs in the treatment of exacerbations in chronic bronchitis does not seem justified

Treatment with physiotherapy caused improvement in the mechanics of breathing in several of the patients, and in spite of the lack of concomitant demonstrable improvement of lung function this might justify physiotherapy in selected cases Clinical improvement was especially noted in cooperative patients with good musculature, whereas scoliosis tended to rule out good results with breathing exercises Clinical improvement occurred within four weeks of treatment Further treatment did not benefit any additional patients, but in the improved patient it may ensure a better fixation of the corrected movement patterns

The positive effect obtained in this series with placebo tablets and placebo-cough mixture cannot be regarded as a placebo effect, strictu sensu as there was no proper control group It is however, the opinion of the authors that the positive responses obtained might well be ascribed to factors such as removal from daily surroundings bedrest proper feeding and nursing, and decreased smoking

Summary

In a strictly controlled study of 38 patients admitted to a medical ward for exacerbations of chronic bronchitis ten patients were treated with physiotherapy for 28 days nine patients received potassium iodide for ten days and nine patients received chloramphenicol for ten days The last ten patients received placebo tablets and placebo mixture The four groups of patients appeared to be fully comparable, being similar in respect of sex, age physical characteristics length and severity of disease as well as of the result of the lung function tests performed An objective evaluation of the effect of treatment was attempted mainly by pulmonary function tests The lung function tests showed no significantly different response to treatment between any of the groups

A clinical improvement in the mechanics of breathing occurred in several patients upon pulmonary physiotherapy but was not correlated to a concomitant improvement in the results of the lung function tests The improvement of the results of the lung function tests noted in several of the patients in each group might be ascribed to non specific factors related to hospitalization

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Triglycerides in Plasma Following Partial Gastrectomy

By

Knut Kirkeby, Kare Overskeid and Inger Bjerkedal

A defect in the digestion and absorption of fat has been demonstrated in from two-thirds to three-quarters of patients following partial gastrectomy with gastro jejunal anastomosis (3, 6) Because of speculations as to a possible link between this disturbance in fat assimilation and a lower incidence of coronary disease (9) in these patients, we previously studied serum lipids in patients who had undergone a Billroth II operation for gastric or duodenal ulcer (4) It was shown that this defect although common in partially gastrectomized individuals does not depress the average serum lipid values Neither the serum concentrations of total cholesterol phospholipids or beta lipoproteins nor the cholesterol/phospholipid ratio were abnormal compared with a material of healthy subjects However slightly higher total lipids in serum were observed in the gastrectomy group, with means of 911 mg/100 ml and 847 mg/100 ml respectively in the two groups The difference between these means was close to statistical significance ($0\,10>p>0\,05$) Since the mean values for two of the major lipid constituents in serum cholesterol and phospholipids were the same it was concluded that a difference in the third, the triglycerides could not be entirely excluded

Italian studies have suggested that gastrectomy influences lipid metabolism other than via fat absorption possibly via disturbances of the clearing mechanism in the blood (7 8)

In one study microscopical counting of chylomicrons in the fasting state revealed higher counts in partially gastrectomized patients than in control subjects (5) In contrast Berkowitz (1) has recently reported a lowering of blood lipids in a selected group of patients who were hyperlipidemic pre operatively

The object of the present investigation has been to study the levels of triglycerides in the plasma of gastrecto-

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mized patients in the fasting state Cholesterol determinations have been included in the study

Material and methods

The gastrectomy group in the study consisted of men aged 40 to 60 years, who had been operated on for a gastric or duodenal ulcer with a Billroth II operation 5 to 10 years prior to the investigation They were selected from the files of the surgical departments II and III, Ulleval Hospital Sixty seven patients were invited to participate in the study and positive answers were obtained from 38 These went through an examination including family and personal history, physical examination B P reading examination of the urine ESR ad modum Westergren Hb determination measurements of height and weight and recording of an ECG with standard leads and unipolar precordial and extremity leads

A personal history of or signs of any major disease a B P reading higher than 140/90 an ESR higher than 15 mm/hr, or pathologic changes in the ECG were considered reasons for exclusion from the study Thirteen of those examined were excluded for the following reasons hematuria 2 hypertension 4 pyelonephritis 2 ECGs suggesting coronary disease 3 and high ESR 2 The remaining 25 patients were admitted to the study Some of them had moderate symptoms from their gastro intestinal tracts but otherwise they had no complaints The range of Hb determinations in the group was from 12 0 g/100 ml to 16 0 g/100 ml

A control material of 25 men was selected from the workers and employees of a factory in Oslo They were chosen from the files of the industrial physician and had for a number of years had annual health examinations with normal findings In the selection it was attempted to match this control material with the gastrectomy group with regard to age smoking habits (smoker or non smoker) and weight/height index The matching of age and smoking habits was strictly carried through, but some small modifications in the matching of weight/height relationship had to be allowed At the end of the investigation, the mean weight/height index according to Broca's formula (weight kg — (height cm — 100)) in the control groups was — 3 8 as against — 4 8 in the gastrectomy group The control group went through the same examination as described for the patient group and the same criteria for admittance to the study were used All subjects in the control group declared themselves to be in good health and without recent illness of any kind The Hb values ranged from 12 2 g/100 ml to 16 1 g/100 ml There was no difference in social standing or occupational activity between the two groups

For the lipid analyses heparinized blood was drawn fasting All determinations were done in triplicate and average values taken

The procedure for triglyceride determination was a modification of that described by Carlson and Wadstrom (2) Total and esterified cholesterol determinations were done by the method of Webster (10)

An attempt was made to evaluate the dietary habits of the gastrectomized patients through interviews by a trained dietician Estimates of quantities were calculated from dietary anamneses and from figures obtained by the 24 hour recall method

Results

In table I, the results of the lipid studies are recorded, with means, standard deviations medians, and ranges

Neither the mean values nor the standard deviations of total or esterified cholesterol differ to any significant degree

The mean triglyceride value is slightly higher in the gastrectomy group than in the control material, 117 and 92 mg/100 ml respectively A non parametric test Wilcoxon rank sum test, shows that the difference is not statistically significant (0 10>p>0 05) (11)

TABLE I Plasma lipids in post gastrectomy patients and in control subjects

		Control material	Post gastrectomy patients
Total cholesterol (mg/100 ml)	Mean	267	271
	SD	40	44
	Median	256	278
	Range	210—363	198—344
Esterified cholesterol (mg/100 ml)	Mean	195	190
	SD	33	46
	Median	189	196
	Range	146—256	90—249
Triglycerides (mg/100 ml)	Mean	92	117
	SD	41	64
	Median	81	100
	Range	30—208	50—340

The estimates of fat intake from the dietary survey in the gastrectomy group revealed great variations, the intakes being from 45 to 199 g day with a median of 108 g/day Thirteen patients regarded their tolerance for fat as very good Eight patients stated that they usually tolerated fats, but that they occasionally experienced some digestive distress following the consumption of certain fatty foods Only four of the patients tolerated fats poorly

Comments

Presumably the two groups in the study are fully comparable except for the previous ulcer disease and the operation performed in the gastrectomy group The participants in the two groups were strictly matched for age and smoking habits, and there was no important difference in weight height index, social standing or occupation Some of the patients in the gastrectomy group had some gastro-intestinal symptoms but otherwise all the subjects were regarded as healthy after a rather extensive general examination

The results confirm the previous finding for total cholesterol values in gastrectomized patients (4) Patients with gastro intestinal symptoms were previously excluded In the present study, patients with mild or moderate dyspepsia were admitted to the gastrectomy group This difference in selection has not affected the results and the study shows that the mean values of total and esterified cholesterol in gastrectomy patients are quite normal

The study has not confirmed the hypothesis that triglyceride values in the fasting state are increased following gastrectomy when compared with those of normal subjects

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Further Studies on Cytomegalovirus Mononucleosis in Previously Healthy Individuals

By

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Recent studies have shown that acquired cytomegalovirus (CMV) infection may sometimes manifest itself clinically as an acute febrile disease with the haematological features of infectious mononucleosis (I M) (1, 10 14, 15, 16) However, the Paul Bunnell (P B) or heterophil agglutination test is negative and exudative pharyngitis and lymph node enlargement are absent The name cytomegalovirus mononucleosis has been proposed for this disease (14) It occurs in previously healthy people as well as after operations in patients who have received large quantities of fresh or relatively fresh blood

The work herein described is a direct extension of the authors basic investigations (10) Some of the information now presented has been already published in a review (11) Our aim has been to elucidate the aetiological significance of CMV in I M like diseases with a negative P B test and to more fully describe the clinical features of CMV mononucleosis Another important objective has been to ascertain how often the CMV complement fixing (C—F) antibody titre changes during the course of various acute infectious diseases

Material and methods

In the present series of 475 patients C F antibodies to the CMV strain Ad 169 were studied at least twice on admission to the hospital and five weeks from the onset of the disease These patients classified according to clinical diagnosis in table 1 were treated at the Aurora Hospital in 1965 —1966 and virological investigations were performed in the Department of Virology University of Helsinki Nine of the patients of group I A and five of those of group I B had been included in an earlier study (10)

The 18 patients with a P—B negative febrile I M like disease (group I B 3) have been the object of various virological microbiological and clinical investigations These patients had not undergone surgical procedures or received blood tranfusions for at least three months before they were taken ill

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TABLE I Clinical classification of the patients in whom the titre of C-F antibodies to CMV was studied in the course of the disease

I	I M or I M like disease	
	A With a positive heterophil agglutination test (titre at least 1 32 after the guinea pig kidney absorption test by the Dawidsohn procedure)	90
	B With a negative heterophil agglutination test	
	1 Anginose or anginose glandular type	13
	2 Glandular type	4
	3 Febrile or typhoidal type	18
II	Acute infectious disease of miscellaneous aetiology	350

Attempts were made to isolate CMV from the urine of eight of these 18 patients and of ten of the patients of group I A Needle biopsy of the liver and an attempt to isolate CMV from the specimen were performed in two patients of the former group Cytological investigation of the urine was undertaken in seven patients of the former group and 15 patients of group I A (table I)

Patients

The haematological criteria on which the diagnosis of I M or I M like disease (group I) is based were relative and absolute lymphocytosis and abundant appearance of atypical lymphocytes for a long period The limit for positivity of the P B test was set at a titre of at least 32 after guinea pig kidney absorption by the Dawidsohn procedure According to the clinical picture the patients with a P B negative I M or I M like disease were grouped into three categories anginose glandular and febrile The anginose type is characterized by exudative pharyngitis, usually accompanied by lymph node enlargement In the glandular type of the disease there is no obvious tonsillitis In the febrile type fever dominates the clinical picture exudative pharyngitis and enlarged lymph nodes are absent All cases with a P B negative glandular or febrile type of the disease were tested for toxoplasma antibodies (Dye test by the Sabin Feldman procedure and C F test) both on admission to hospital and five weeks after the onset of the disease A significant rise in titre occurred in two patients with the glandular and in two with the febrile type Nevertheless these patients are included in the group of I M like diseases (table I), since the clinician cannot make the diagnosis until a relatively late phase of the disease Group II acute infectious diseases of miscellaneous aetiology includes 185 patients with aseptic meningitis or encephalitis 45 with pneumonia 42 with infectious hepatitis, 21 with measles, 15 with salmonella infections and 12 with mumps

Complement fixation test

In human embryonic fibroblasts C F antigens were prepared from the CMV strains Ad 169 Davis and two new strains previously isolated in Helsinki from adults suffering from CMV mononucleosis Antigens were prepared and C F tests performed as described previously (10)

Virus isolations

Sixty ml of urine was mixed with an equal volume of Eagle s minimum essential medium containing 0 2% bovine serum albumin The mixture was centrifuged at low speed for 15 min and the sediment discarded The supernatant was spun in a Spinco ultra centrifuge for two hours at 35 000 rpm Each pellet was resuspended in 0 5 ml of the maintenance medium (Eagle s minimum essential medium with 3 % calf serum) Human embryonic fibroblasts in Falcon plastic flasks or tissue culture tubes were inoculated with

from 0 5 to 2 ml and with 0 2 ml of the suspension respectively The maintenance medium was changed on the following day and subsequently at intervals of five days Inoculated cultures were examined twice a week for six to eight weeks Positive cultures were passaged by scraping off the cells with a pipette and transferring them to new cul ture flasks and one Falcon plastic flask was stained with haematoxylin eosin after fixation with Bouin s solution

Cytological examination of the urine

About 200 ml of fresh urine was filtered through several Millipore filters (pore size 5 microns) The cellular material attached on the filters was fixed in 96 % alcohol and stained according to Papanicolaou Slides were scanned and all unusual cells marked with an ink dot and studied at high magnifi cation

Electron microscopy

For electron microscopy the virus was grown in primary human embryonic fibroblasts in 10 ml carrel bottles After three weeks cultivation the cells were fixed in the bottles with 3 % glutaraldehyde (25) The cell sheets were removed from the glass with a razor blade and transferred to 1 % osmium tetroxide for one hour Fixation was followed by dehydration through a progressive ethanol series and embedding in Epon 812 (17) Sections were cut with a Porter Blum MT 2 ultramicrotome and stained with either ura nyl acetate (30) or lead citrate (22) Elec tron micrographs were taken with a Siemens Elmiskop I or a Philips 200 at actual magnifications of 4 000 to 40 000

Results

Virological Studies

Cytomegalovirus antibodies

A significant (4 fold or greater) in crease in C F antibodies to CMV strain Ad 169 during the course of the disease was demonstrated in 13 of the 18 pa tients with a febrile, P B negative I M like disease (group I B 3 of table I) Since five of these cases of CMV mono-nucleosis have been reported earlier (10), only the eight most recent cases will be discussed here No marked dif ferences could be found in the titres against antigens from different strains of the CMV In five cases no antibodies could be demonstrated in the first serum sample (table II) In two of these (nos 5 and 8) antibodies developed so slow ly that none could be demonstrated at 14 and 20 days, respectively, after the onset of the fever In all the 13 cases mentioned above a serum sample was also studied 1/2 to 1 year after the ill ness By that time, the titres had fallen to levels of 4 to 64 Case 8 which will be discussed later, is exceptional in that active infection with toxoplasma was concurrently present In the other 12 cases toxoplasma antibodies were either lacking or present in low titre

In five of the 18 cases of group I B 3 no change in the CMV C F antibody titre could be demonstrated These pa tients were adults, in whom the dura tion of the fever was 5 to 20 days in all of them laboratory tests showed affec tion of liver function and atypical lymphocytes were abundant for a long time In two of these no CMV anti bodies could be demonstrated and in two cases the titre was <16 and did not change No toxoplasma antibodies could be demonstrated in these four cases The P B titre was in one case 8 in the other three <4 In the 5th case infection with toxoplasma was demonstrated On the 9th day after the onset of the disease

scattered through the nucleoplasm with condensation along the chromatin like material Intranuclear membrane bound inclusions containing numerous double coated virus particles were also seen (fig 1) In the cytoplasm the viruses were found either free bound to membranes or in around electron dense lysosome like inclusions Most cytoplasmic particles were double coated but single coated ones were also always present All these findings as well as the particle size (90 to 100 mu) correspond fairly well with earlier electron microscopic observations concerning the CMV (18 26) but other viruses of the herpes simplex group naturally cannot be excluded

On the basis of the typical cytopathic effect in tissue culture as well as electron microscopic and serological findings these agents were regarded as cytomegaloviruses In every instance C F antigens were prepared from the infected cell cultures These antigens were tested against human sera previously known to give positive and negative reactions respectively to Ad 169 and Davis strain antigens and were found to give closely similar results

Attempts were also made to isolate virus from the liver biopsy specimens of two patients In case 5 a cytopathic agent with the characteristics of CMV was isolated from the liver tissue as well as from the urine three weeks after the onset of the disease

Unsuccessful attempts to isolate virus were also made in ten cases of P B positive I M and in three cases of P B negative I M without a rise of CMV antibodies

Clinical histological and cytological features in patients with cytomegalovirus mononucleosis

In the following only the eight patients listed in table II will be described since five out of the 13 cases have been reported previously (10)

Table III shows the sex and age distribution of these patients No epidemiological connection between the cases could be traced The 13 patients with CMV mononucleosis constitute 9 % of the total number of patients treated for I M or I M like disease in the Aurora hospital in 1965—1966 Of the 328 I M patients treated in the same hospital in the five preceding years the P B negative febrile cases constituted 4 3 % (9) although this group obviously also includes diseases of other aetiology as has already been implied The results give the impression that during 1965 the disease concerned occurred more frequently than usual and that the great number of cases diagnosed was not exclusively due to the greater interest in the matter Thus no new cases were diagnosed during the second half of 1966

The fever lasted for 3 to ?? days and was often irregular The general condition of the patients was surprisingly good and their subjective symptoms relatively slight Six patients had mild ache in the head neck or extremities but not a single one had respiratory or gastrointestinal symptoms A rubelliform rash appeared on the 8th day in case 7 The rash covered the trunk and extremities and lasted for several days In no case was tonsillitis or lymph node enlargement found A clearly palpable spleen

TABLE III Clinical and laboratory data in patients with CMV mononucleosis

Case no	Age (yrs)	Sex	Duration of fever (days)	Highest fever (C)	Highest ESR (mm/hr)	SGOT (maximal value according to Reitman & Frankel) (20)	Thymol turbidity test (maximal value in MacLagan units)	Titre of cold agglutinins (first and highest titres)	Complications
1	18	♀	11	38 6	17	47	5 7		Myocarditis
2	36	♂	9	38 5	21	123	9 2	4 16	
3	65	♀	14	38 2	27	35	10 0		
4	26	♀	3	38 3	14	64	6 5		
5	66	♀	19	39 6	40	80	4 5	4 32	Myocarditis
6	54	♀	18	40 4	37	54	3 2	4 4	
7	19	♀	22	39 7	12	44	5 0	16 512	Exanthema
8	19	♂	22	40 0	10	66	7 9	4 512	Concurrent infection with toxoplasma Myocarditis

was found in only one patient The elevation of the ESR was relatively slight In all the cases liver function tests (SGOT, thymol turbidity test) gave abnormal results, but none of the patients was icteric

Clinically, three cases were complicated by reversible ECG changes apparently due to myocarditis In case 1 the T wave was isoelectric or biphasic in leads II, aVF V_5 and V_6 and negative in V_4 in case 5 the T wave was negative in leads I II, a VL, a VF and in leads V_1—V_6 In addition a transient ly negative T wave was noted in the V_2—V_3 leads in case 8, a patient who had both active CMV and toxoplasma infections It is thus impossible to decide the extent to which each of the concurrent infections was responsible for the clinical and haematological symptoms This patient had been treated in the same hospital eight months earlier for an anginose glandular, P B positive I M At that time no CMV or toxoplasma antibodies could be demonstrated Nine days after the onset of his current disease the dye test and C F test for toxoplasma were 16 and 4 respectively rising to 1 024 and 256 three weeks after the onset of the disease

Table IV shows the leucocyte differential count at the time of maximal leucocytosis As in the cases reported earlier there was often leucopenia at the beginning of the disease and no atypical

TABLE IV Differential counts of leucocytes (in percentages) at the examination revealing the highest number of leucocytes in patients with CMV mononucleosis

Case no	No of leucocytes (/mm³)	Rod nucleated leucocytes	Segmented leucocytes	Eosinophils	Basophils	Monocytes	Atypical lymphocytes	Other lymphocytes
1	8 300	—	25 0	4 0	1 0	5 0	16 0	49
2	12 100	3 0	20 5	10 0	—	7 5	12 0	47
3	11,300	—	26 0	1 0	—	2 5	22 0	48 5
4	10 000	3 0	20 0	0 5	—	4 0	10 0	62 5
5	15 300	4 5	16 0	1 5	0 5	2 0	24 0	51 5
6	12 100	3 5	22 5	2 5	—	3 0	16 0	52 5
7	15 700	4 5	16 0	1 5	—	3 5	19 0	55 5
8	18 700	2 0	7 5	2 0	1 0	1 5	55 0	31 0

lymphocytes could be found Occasionally the haematological changes, which were morphologically indistinguishable from I M, did not appear until 1—2 weeks from the beginning of the disease The peak leucocytosis was often found relatively late in the disease when the fever was already subsiding or had abated Occasionally the relative and absolute atypical lymphocyte count was already declining at that time Only typical Downey cells have been classified as atypical lymphocytes in the table, though actually a high percentage of the other lymphocytes were large and abnormal, too Using other criteria (8) many of these could also be regarded as atypical lymphocytes As in our earlier studies, the abundance of atypical lymphocytes was found to persist for at least two weeks Nothing abnormal could be detected in the thrombocytes or erythrocytes

Serological test for syphilis, salmonella, leptospira, Mycoplasma pneumoniae and Listeria monocytogenes were negative The result of the P B test remained <4, except for cases 4 and 8, patients who earlier had had a typical P B positive I M and exhibited titres of 8 The antistreptolysin and antistaphylolysin titres were in all cases within normal limits Blood cultures for bacteria were performed in six cases, with negative results A significant rise of the cold agglutinin titre according to the method of Feller and Hilleman (5) was found in four out of five patients examined (table III)

Paper electrophoresis was carried out in seven cases during the febrile stage of the disease but no systematic studies were performed Only in case 8 was an increased γ fraction noted In one case (case 8), immunoelectrophoresis revealed an M component like deviation of the Ig G line and in another case (case 3) of the Ig M line (29)

Needle biopsy of the liver tissue was done in two cases (cases 1 and 5) ten days and three weeks after the onset of the disease, respectively In the latter case a moderate mononuclear, inflammatory infiltration was seen in the portal

areas The sinusoids contained a few mononuclear cells Parenchymal cells showed fatty degeneration and often vacuolization of the nucleus In the parenchyma small focal infiltrates consisting of mononuclear cells, occasional histiocytes and degenerated liver cells could also be detected No intranuclear inclusion bodies were seen In the other case only slight inflammatory changes occurred in the liver tissue

Cytological examination of the urinary sediment was performed in seven patients with CMV mononucleosis In one case (case 5), a few large, atypical cells were encountered which had a fairly abundant granular cytoplasm with occasional acidophilic inclusions The cells contained 1 to 3 medium sized pyknotic, round nuclei, each with a large single acidophilic inclusion body No atypical cells with intranuclear inclusions could be detected in the urinary sediment of 15 patients with P B positive I M

Discussion

From our present and previous studies and some observations made by other authors (1, 2, 4, 16), it is apparent that cytomegalovirus (CMV) mononucleosis occurs in many countries and that it is not particularly uncommon During the last two years the incidence of this disease has been 9 % of all patients treated at the Aurora Hospital with the diagnosis of infectious mononucleosis (I M) or I M like disease Since subclinical infections with CMV are common (3, 24 27 31) there is always a possibility that a patient may contract a CMV infection for the first time in his life concurrently with some other disease In the case of an acute infectious disease, the likelihood of such a coincidence would appear to be small In the present study, among 350 patients suffering from infectious disease of miscellaneous aetiology, only one was found who initially had no demonstrable antibodies to CMV but in whom the titre rose in the course of the disease Moreover, the possibility has to be taken into consideration that in this case, as in a case of the Guillain Barre syndrome published separately (13), the CMV infection had played a role in the aetiology of the neurological disease

CMV was isolated from the urine of three patients suffering from CMV mononucleosis some three weeks after the onset of the fever In a case published elsewhere (16), the virus was isolated from the urine and saliva four weeks from the onset of the disease, and in a case of similar disease after open heart surgery (15) it was isolated from the urine at 40 days Further study is required to ascertain the onset and duration of virus excretion in the urine of patients with CMV mononucleosis A large scale study in London revealed not a single CMV excretor among 100 healthy children aged 6 to 10 years or among 400 older children and adults (16)

With one exception (10) all confirmed cases of CMV mononucleosis have so far occurred in adults On the other hand according to some authors (21 23) the proportion of children may amount to at least one third among patients who after open heart surgery

contract a syndrome resembling I M, an illness which in many cases at least is due to CMV according to recent investigations (14, 15) Our studies have been predominantly concerned with adults, this fact may naturally have influenced the results However, the possibility also has to be taken into account that — as in some other viral infections, e g poliomyelitis and infectious hepatitis — the older the age group primarily infected, the higher the proportion in which the infection is followed by clinical disease Moreover, it is well to remember that the younger the child, the more difficult is the haematological differential diagnosis, since in infancy and early childhood there is a tendency to lymphocytosis and a prevalence of large lymphocytes

Good general condition and scarcity of subjective and objective symptoms seem to be characteristic of the clinical picture of CMV mononucleosis In mild cases in which the fever is of short duration the clinical diagnosis is very difficult, since haematological changes are often not to be found until a relatively late stage of the disease From this and our previous study (10), a rise of cold agglutinins seems to be a common feature *in CMV mononucleosis This interesting* phenomenon deserves further study Liver involvement seems to be a characteristic feature of CMV mononucleosis The disease may simulate infectious hepatitis if it is accompanied by jaundice (16, 19) Further study is required to elucidate the possible importance of CMV mononucleosis in causing chronic diseases of the liver and spleen in adults There is some evidence that an acquired CMV infection may cause chronic liver disease in children (6, 7, 12) As in the case of hepatic and acute neurological diseases (13), acquired CMV infection should be taken into consideration in the aetiology of myocarditis

A simultaneous rise in the titre of CMV and toxoplasma antibodies in the course of the disease was demonstrable in one of the eight patients with CMV mononucleosis Naturally, it is impossible to determine to what extent the symptoms in the above mentioned case were due to CMV or to toxoplasma infection A high titre of CMV antibodies was found in one other patient with I M-like haematological findings and a significant rise of toxoplasma antibodies during the disease This patient has not, however, been included in our series of patients with CMV mononucleosis, since a high titre of CMV antibodies was already present at the onset of the febrile disease In both these cases with concurrent CMV and toxoplasma infection, immunoelectrophoresis showed an M component like deviation in the Ig G line These changes will be described in detail elsewhere (29) Our observations may be indicative of some special re*lationship between CMV and toxo*plasma infections The one may in some way lower resistance to the other It is well known that these two infections have many epidemiological and clinical features in common Here it may be mentioned that cytomegalo inclusion disease has been found in patients who died from pneumonia due to Pneumocystis carinii which, like toxoplasma, is a protozoan (28, 32)

In a previous study (10) attention was paid to the observation that a high titre of C F antibodies to the CMV at an early stage of the disease without a subsequent rise was found in two out of 19 patients with a P B positive I M In one of these patients the disease was of glandular type with jaundice This observation is of special interest since in our present series, in the group with P B positive I M the only patient who had a high antibody titre to the CMV in the first serum sample, had the febrile type of the disease also associated with jaundice Further study is required to elucidate the pathogenesis of the clinical disease and the cause of the positive P B test in these icteric cases

From our observations it is apparent that — besides toxoplasma and CMV — other aetiological agents may be the cause of a febrile syndrome haematologically resembling I M without a positive heterophil agglutination test It is noteworthy that in our cases of this category, a feature common to CMV and toxoplasma mononucleosis as well as to P B positive I M is the frequency of liver damage

Summary

A report is given on eight further cases of cytomegalovirus mononucleosis in previously healthy individuals raising the total number of such cases seen by the authors to thirteen A significant rise of complement fixing (C F) antibodies to the cytomegalovirus (CMV) strain Ad 169 was shown during the disease In three of the four patients most recently examined, CMV was isolated from the urine and in one case from a liver biopsy specimen as well All the patients were adults Fever dominated the clinical picture Haematologically, the disease was indistinguishable from typical Paul Bunnel (P-B) positive infectious mononucleosis (I M), but the heterophil agglutination test was negative and exudative pharyngitis and enlargement of the lymph nodes absent Liver function tests gave abnormal results in all cases Myocarditis was diagnosed in three patients A concurrent infection with toxoplasma was demonstrated in one case

On the other hand, there were five patients with a febrile, P B negative, I M like disease in whom no rise in the CMV antibody titre was demonstrable during the disease In one of these, there was a significant rise in antibodies to toxoplasma

C F antibodies to the CMV were also studied in a large series of patients comprising 90 with a P B positive I M , 17 with an anginose or glandular type of P B negative I M and 350 with acute infectious diseases of miscellaneous aetiology A significant rise of C F antibodies to the CMV was found in only two patients of the last group

Acknowledgements

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Acute Lower Respiratory Illness in Elderly Patients with Respiratory Syncytial Virus Infection

By

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Respiratory syncytial (RS) virus has a wide geographical distribution (1, 4, 8, 9, 13, 24–26, 29–30) and is a common cause of acute respiratory illness during infancy and early childhood (2, 5, 6–7, 12, 16, 17, 21). In adults RS virus is said to cause only upper respiratory illness (11, 15, 28) and generally to represent reinfection (11, 15). Also acute exacerbations in chronic bronchitis in adults may sometimes be caused by RS virus (3, 19).

This paper reports the occurrence of serologically proven RS virus infection in people above 55 years of age with acute respiratory illness. The aetiological role of the RS virus infection is discussed and the fact that mixed infections were fairly often demonstrated is taken into account.

Material and methods

A long term study of the aetiology of acute respiratory illness in children and adults admitted to the hospital for Infectious Diseases in Stockholm has been in progress since 1963. In a preliminary analysis of results for the period Oct. 1963 — June 1964 it was found that not only young children but also middle aged and older people had RS virus infections (21). This observation led us to continue to investigate this aspect in material collected later.

Up to June 1966 18 patients over 55 years of age presented significantly rising complement fixing (CF) antibody titres against RS virus.

Virological studies. No attempt at virus isolation was made in the long term study mentioned above. For the serological study blood was sampled on admission and ten and 21 days later (table I). Paired sera were tested for CF antibodies against adenovirus, influenza A and B, parainfluenza 1, 2 and 3, mumps and RS viruses and against a psittacosis antigen. The methods used have been described elsewhere (10, 20, 23, 25, 27). A four fold rise in titre was considered as significant. Neutralization tests (NT) against RS virus were also carried out in two cases.

Mycoplasma studies. Attempts to isolate Mycoplasma pneumoniae (MP) from sputum and throat swabs were made on admission (table I). Paired sera were tested for CF antibodies against MP antigen, again a four

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TABLE I Schedule for taking samples For patients admitted late in the afternoon the start of schedule was postponed for one day Depending on the week end situation small deviations were tolerated after the first three days

Day in hospital	Throat swab	Naso-pharyngeal swab	Nasal swab	Expectorate (if produced)	Blood sample
1	B	B+MP	B	B+MP	S
2	B		B	B	
3	B	B	B	B	
7	B	B	B	B	
10					S
14	B	B	B	B	
21	(B)	(B)	(B)	(B)	S

B = bacterial culture
MP = culture for Mycoplasma pneumoniae
S = serological investigation

TABLE II Age distribution of patients with a significant (≥4 fold) titre rise in the CF test against RS virus compared with the age distribution in the whole material of acute respiratory illness investigated from Oct 1963 up to June 1966

Age	No of cases	
	With significant rise	Tested
< 1	3	12
1—2	2	32
3—6	4	51
7—14	1	84
15—19	4	97
20—29	0	150
30—39	0	67
40—49	3	85
50—59	2	99
60—69	5	78
>70	11	138
Total	35	893

fold rise in titre was considered as significant Methods used are described elsewhere (25)

Bacteriological studies Nasopharyngeal nose and throat swabs as well as sputum when available were cultured for bacteria on admission and also later on (table I) Pneumococci β streptococci Staphylococcus aureus and Haemophilus influenzae were especially looked for As far as the amount of serum permitted paired sera were examined for antipneumolysin (APn), antistreptolysin (AS) antistaphylolysin (ASta) and CF antibodies against Haemophilus influenzae (AHI) A reaction was considered as significantly changed when antilysin titre rose more than two fold and CF antibody titre four fold The methods for the bacteriological investigation are likewise given in previous reports (20, 27)

Clinical studies All patients were examined by one of us (H F or G S) X ray investigation of the chest was carried out on admission and several times afterwards The radiograms were examined by Dr P O Gribbe Routine laboratory examinations were carried out including ESR Hb white cell count and urine analysis

TABLE III Results of CF test and NT against RS virus in 18 patients above 55 years of age (NT carried out in two cases only) Cases 1—6 had bronchitis and cases 7—18 had bronchopneumonia

			CF antibodies		
Case no	Age	Sex	Serum I	Serum II	Serum III
1	63	♀	4	64	64
2	58	♀	2	4	32
3	74	♀	2	16	—
4	76	♀	256	1024	—
5	74	♂	<4	128	128
			NT2	NT32	—
6	82	♀	2	64	64
7	80	♀	<16	256	128
8	61	♂	< 4	64	64
9	62	♀	4	>256	—
10	58	♀	4	>512	256
11	88	♂	4	64	—
12	79	♀	32	256	128
13	66	♀	< 2	32	—
14	68	♀	< 2	4	8
15	84	♂	8	>256	—
16	74	♀	16	>256	—
17	80	♂	< 4	16	32
18	77	♀	16	>256	>256
			NT4	—	NT32

— = not done

Results

Virological findings The predominance of RS virus infections in young children and people 70 years of age or more is illustrated in table II Detailed results of the CF and neutralization tests against RS virus in the patients above 55 years are given in table III A rise in neutralizing antibody titre against RS virus accompanied the rise in CF antibody titre in both of the cases studied (cases 5 and 18)

Only in one case there was a titre rise against any of the other viral antigens used In a man of 80 years (case 17) the CF antibody titre against influenza A rose from < 8 to ⩾ 256 In other cases the titres against viral antigens other than RS virus were generally low (⩽ 8)

Mycoplasma findings In no case was MP isolated, nor was any rise in CF antibody titre against MP antigen demonstrated the titres being generally low

Bacteriological findings Before admission to the hospital six patients had received chemotherapeutic drugs or antibiotics (table IV)

Potentially pathogenic bacteria were found in seven cases In two cases a bacterium not present on admission was

TABLE IV Result of bacteriological investigation in 18 patients above 55 years of age with proven current RS virus infection Cases 1—6 had bronchitis and cases 7—18 had bronchopneumonia

Case no	Age	Sex	AS	ASta	APn	AHI	Bacteria isolated
1	63	♀	—	—	—	—	
2	58	♀	—	—	—	*	H influenzae Pneumococci[1]
3	74	♀	—	—	—	—	
4	76●	♀	—	—	—	—	
5	74	♂	—	—	—	—	Pneumococci
6	82	♀	—	*	—	—	Pneumococci
7	80	♀	—	—	—	—	
8	61●	♂	*	—	—	—	
9	62●	♀	—	—	—	—	
10	58	♀	—	—	*	—	
11	88●	♂	—	—	*	—	
12	79●	♀	—	*	*	—	Aerobacter[2]
13	66	♀	—	—	—	Sera out	H influenzae[1]
14	68	♀	—	—	—	*	H influenzae
15	84	♂	—	—	*	—	
16	74	♀	—	—	—	Sera out	
17[3]	80●	♂	*	*	—	Sera out	H influenzae
18	77	♀	Sera out	Sera out	Sera out	Sera out	

AS = antistreptolysin titre ASta = antistaphylolysin titre APn = antipneumolysin titre AHI = CF antibody titre against Haemophilus influenzae
* = significant titre rise
— = no titre rise
● = treated with antibiotic before admission
[1] Bacteria isolated one week after hospitalization but not at the admission
[2] Pure culture in sputum at admission
[3] This case had also a significant rise in CF antibody titre against influenza A

isolated after one week in the hospital

As is seen from table IV eleven rises of antibody titres against bacterial antigens were demonstrated In two cases where AHI rose the corresponding organism was recovered

Infection with RS virus occurred together with one bacterial species in seven patients, and together with two bacterial species in two patients

In eight cases no antibacterial reactions were observed although in two of them no AHI test was performed, moreover, in three of them all titres were below conventional upper limits for their normal variation (20) All of the latter belonged to the bronchitis group

Epidemiological and clinical findings The ages of the patients ranged from 58 to 88 years (table III) Five were male

and 13 female. Most of the older patients lived alone and had few contacts with other people. Only two of the 18 patients knew of any contact with a person suffering from acute respiratory illness during the last week before onset of their own disease. The patients were admitted to the hospital in the winter months of 1964, 1965 and 1966.

Chest X-rays showed bronchopneumonia in 12 of the patients. The clinical diagnosis was acute bronchitis in the other six patients, only one of whom had a previous history of chronic bronchitis. The highest temperature during the illness was ⩾ 39.0° C in 16 cases, slightly above 38.0° C in the remaining two cases. The duration of fever (⩾ 37.4° C) was in five cases 4 to 7 days, in seven cases 8 to 14 days, in two cases 15 to 21 days, and in four cases more than 21 days. All the four cases with fever of more than three weeks' duration had mixed infections. Three patients with bronchitis had fever for less than eight days, but this was likewise the fact in two cases with pneumonia.

All the eight patients with RS virus infection lacking evidence of simultaneous bacterial infections had a cough which sometimes was hard and hoarse. Muscular pain and headache were frequent. Conjunctivitis and lymphadenopathia seldom occurred. Only one patient had diarrhoea. Two cases had rhonchi and two had rales, radiologically patchy changes of the lungs were present in four patients. The ESR varied from 24 to 98 mm/hr. In only one case was the white blood cell count above 10 000/mm³ (highest value 12 800/mm³). There was a slight rise of the polymorphs (⩾ 70 %). Haemoglobin was normal. The urine finding was abnormal in only one case with cystitis.

The clinical character of cases with mixed infections did not differ appreciably from the above.

Discussion

The majority of reports concerning RS virus infection have shown that this virus is one of the most important causes of acute respiratory illness in infants and young children (2, 6, 11, 14, 16). In early childhood it may cause not only upper respiratory illness, but also diseases of the lower respiratory tract such as bronchitis and bronchopneumonia (16, 24). This ability of RS virus to cause lower respiratory tract illness has been evident especially in hospitalized infants and young children (7, 14, 17).

In older children and adults the RS virus has been thought to cause only mild upper respiratory disease (11, 12, 15). However, in 1963 Sommerville reported a retrospective serological study in which he found rising CF antibody titres against RS virus in 96 paired sera, 85 % of them obtained from adults and half of them from patients over the age of 50 (19). Half of the adults had suffered from bronchopneumonia and the other half had acute exacerbations of chronic bronchitis. No significant antibody titre rise in the CF test against RS virus was observed in a control group collected from adults and from children with non-respiratory illness during the period of the study. Carilli et al. (3) have also reported serologically proven RS virus infection in eight adults with

chronic bronchitis In our material, as is seen from table II, the cases with RS virus infection proven by significantly rising titres were found chiefly among the youngest children and among old adults, whereas they were uncommon in ages from 20 to 40 The 18 patients presented here were all over 55 years of age and most of them over 70 They were admitted to the hospital during winter months It is well known that RS virus is often prevalent in the community during that season causing peak like outbreaks of acute respiratory illness among infants and young children (6, 9)

In Sweden (10), as in other countries (11, 28), children are known to come in contact with RS virus very early It is also reported from other countries that a high proportion of persons in all age groups have neutralizing and CF anti bodies against RS virus (28)

Such data and the fact that reinfec tions with RS virus are known to occur (11, 15) suggest that the RS infections of old people which are associated with clinical symptoms from the lower respi ratory tract may be reinfections which appear when the immunity is waning

Like infants and small children hospi talized for RS virus infections, our group of older people quite often had broncho pneumonia At least nine of the patients had a bacterial infection at the same time as their RS virus infection Seven of them belonged to the pneumonic group This may be interpreted to indicate that the bacterial infection rather than the RS virus infection itself caused the bronchopneumonia According to our previous experiences (22, 23 27) as well as those of other authors (12, 18, 30, 31), mixed infections with more than one virus or with virus and bacteria are not uncommon in hospitalized patients with acute respiratory illness Continued studies may indicate whether broncho pneumonia in old people can be caused by RS virus alone or whether a bac terial component is necessary

Our study has shown that during the winter months, when RS virus is known to be circulating in the community, the possibility of RS virus infection should be borne in mind also in older people with acute lower respiratory illness The clinical picture of such RS virus infec- tion does not differ from that of diseases caused by other viral or bacterial agents The diagnosis of RS virus infection must therefore be based not on clinical but on virological findings

Summary

A long term study of the aetiology of acute respiratory illness in children and adults admitted to the Hospital for In fectious Diseases, Stockholm has been in progress since 1963 During the winters of 1964 to 1966, 18 patients aged 55—88 years most of them over 70 had sero logically proven RS virus infection asso ciated with acute lower respiratory ill ness six had bronchitis and 12 had bronchopneumonia The aetiological role of RS virus infection in the di eases of these patients is discussed in the light of the results of the serological tests not only against viral antigens other than RS virus but also against Mycoplasma pneumoniae antigen and bacterial anti gens (streptococci, staphylococci pneu

mococci and Haemophilus influenzae) Seven out of nine patients with serological evidence of coexisting bacterial infection had bronchopneumonia

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Acute Right Heart Failure During Treatment with Epsilon Amino Caproic Acid (E-ACA)

By

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It has been debated whether E-ACA, which has a strong anti fibrinolytic activity could inhibit the fibrinolysis necessary for dissolving formed clots thereby contributing to thrombus formation (12) Arterial thrombosis has been noted by Naeye (11) and Andersson (1) during treatment with E ACA given after prostatic surgery and in uremic patients suffering from carcinoma of the prostate It is uncertain whether E ACA contributes to the fatal outcome in these cases There have been no reports of accelerated coagulation of blood with thrombus formation

The patient now reported developed during E-ACA therapy acute right heart failure and showed a peculiar pattern of some of the coagulation factors

Methods

Laboratory tests

Bleeding time was determined according to Duke (3)

Coagulation time was determined in glass tubes by a modification of the Hedenius method (7) and in plastic tubes by the method of von Franken and Zetterqvist (5,

Fibrinogen level was determined according to Bergstrom et al (2)

Platelets were counted according to Kristensson (8)

Thrombotest was performed according to the method of Owren (13)

Case report

A female aged 47 Diabetes mellitus was known since 1937 In Nov 1965 a carcinoma of the right mammary glands was found The patient was treated with X ray cobal (4 000 r, and cortisone aceta e 50 mg daily In Feb 1966 metastases in her vertebral column were noted No metastases were found in lungs at this time In March 1966 bilateral oophorectomy was made On 1st May 1966 blood appeared in the urine Cystoscopy revealed a necrotic area low in the bladder The woman was treated with 20 g E ACA intravenously daily from 2nd to 6th May and 100 mg cortisone from 2nd May until she expired During these days

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TABLE I Bleeding time coagulation time coagulation time in plastic tubes and thrombotest during and after treatment with E ACA

Date	Bleeding time	Coagulation time: Hedenius	Coagulation time: In plastic tubes	Thrombotest
22 2				90
2 5	1 15	5 55		
3 5	2 30	4 05	14 30	35
4 5				30
5 5	1 25	7	12	24
6 5	10 35	7 10		20
7 5	4 45	5 55		40
9 5	3 20	7 10	19 30	90
11 5	2 10	4 50		40
13 5	1 50	5 20		
25 5				85

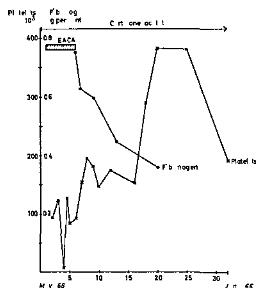


Fig 1 Blood platelet number x——x and fibrinogen concentration o——o during and after treatment with E ACA

she developed dyspnoea and hyperpnoea and became cyanotic she got nausea and felt tenderness as well as distension of the abdomen Her jugular veins were distended Right ventricular strain was noted on her ECG X ray of her lungs on 6th May showed bilateral hydrothorax and a few large metastases up to 2 cm When the E ACA was withdrawn on the evening of 6th May she felt better within a few hours The nausea and distension of the abdomen disappeared as did the dyspnoea cyanosis and distension of the jugular veins, and her ECG reverted towards normal Blood therapy was given on the 7th and 8th without complications During the next two weeks she felt better and better In June an X ray check of her spine, ribs and lungs showed widespread metastasis but no hydrothorax

The patient died on 25th July 1966 Autopsy showed an adenocarcinoma of her right mammary glands with massive metastazation in her lungs ribs spine and liver Subacute infarctions were noted in her heart lung and liver tissue

Platelet counts were performed almost daily during the month of May The results are shown in fig 1 Low platelet counts were noted during the whole E ACA treatment period with an increase when the therapy was stopped Coagulation time measurements were within normal limits Only three determinations of the coagulation time in plastic tubes were performed Two of these taken during E ACA therapy showed values at the lower limit of normal The third done a few days after the E ACA

therapy was withdrawn, gave a somewhat higher value

The thrombotest values were low during E ACA therapy High values of fibrinogen during E ACA therapy were noted The fibrinogen content decreased steadily after the E ACA therapy was withdrawn

Discussion

The close time relation between the administration of E ACA and the signs of pulmonary arterial obstruction suggests a casual relation This is supported by the disappearence of symptom after the withdrawn of the drug The improvement was accompanied by a rise of thrombotest and platelets suggestive of correction of a deficiency state Such a state is known to exist when intra vascular coagulation consumes coagulation factors The high fibrinogen content found during the period of pulmonary arterial obstruction seems puzzling, but it is well known that fibrinogen may be increased in cases with malignant tumours and infection Besides the malignancy this patient had a febrile urinary infection

The therapy in this case deserves special note Cortisone acetate was given continuously from Feb 1966 till the fatal outcome in July Right heart failure appeared during this treatment but could not be caused by it, as the symptoms disappeared during the treatment

E ACA is a valuable therapeutic agent in conditions with increased fibrinolytic activity in blood and urine (12) E ACA, being an inhibitor of plasminogen activation may be a potentially dangerous compound particularly in the presence of an underlying thrombotic state (4 6 9, 10) But according to Nilsson et al (12) there is so far no evidence that E ACA can inhibit lysis and thereby cause a defibrination syndrome with intra vascular coagulation

In the cases of Naeye (11) and Andersson (1) renal function was impaired and the concentration of E ACA in plasma presumably abnormally high (12) In the present case however, renal function was normal In conditions when intravascular coagulation is suspected a combination of E-ACA therapy with heparin seems to be logical (1, 12)

Summary

A case of mammary carcinoma with haematuria developed symptoms of acute right heart failure during treatment with E ACA Thrombocytopenia and low thrombotest values were noted When the E ACA therapy was stopped the symptoms disappeared and the coagulation factors became normal

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Splenic Tumor Post Partum

By

HANS MELIN, ERHARD ZELCHNER and HANS ÅBERG

The purpose of this article is to review some differential diagnostic problems that arose in a young female post partum We will discuss what caused a rapidly growing tumor below the left costal arch, with special regard to the late pregnancy and puerperal period

Case report

The patient was as 23 year old female who worked as an assistant in a research laboratory in Umeå She had earlier been well and was pregnant for the first time On May 3 1966 she delivered a full term male infant of weight 3 260 g and body length 49 cm The pregnancy and the delivery were without complications On May 6 the third day after the delivery the patient suddenly became sick *She developed a feeling of pressure and discomfort in the left upper quadrant of the abdomen* The next morning she woke up with fever and chills and a temperature of 39 0 C Physical examination revealed a healthy looking young woman in moderate distress The pertinent findings were abdominal A firm regular tumor extended 5—6 cm below the left costal arch and it also crossed the midline of the abdomen The left lower rib cage seemed to be more prominent than the right The abdominal tumor was not pulsatile No bruit or friction rub was heard There were no palpable lymph nodes and the liver was not enlarged The patient was transferred to the medical ward The presumptive diagnosis was thrombosis or thrombophlebitis of the splenic vein or a splenic artery aneurysm

The laboratory data on admission to the medical ward on May 7 were Hb level 13 0 g/100 ml hematocrit value 36 % white blood cell count 12 200 with 84 % polymorphonuclear leukocytes and ESR 36 mm/hr Serum bilirubin prothrombin level and platelet count were all within normal limits Clotting and bleeding time were normal A bone marrow biopsy showed an active marrow with unspecific reactive changes in the myelopoiesis No signs of leukemia were noted On X ray the flat plate of the abdomen showed a big tumor which probably *comprised an enlarged spleen* Another flat plate three days later revealed that the tumor had increased in size

In the medical ward she felt well initially She had no fever since May 7 A celiac angiography was planned A few hours prior to the scheduled angiography on May 12 the patient developed intense pain in the epigastric area The pain increased with deep respirations She also developed tachycardia and profuse sweating No drop in the B P

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was recorded The sudden impairment required an immediate operation and the presumptive diagnosis was a ruptured aneurysm of the splenic artery

The exploration revealed an enlarged spleen with a cyst of the size of a child's head Extirpation in toto was difficult and therefore the cyst was punctured frontally It contained about 950 ml of a yellowish brown fluid The weight of the spleen, including cyst and fluid was 2,750 g

The microscopic examination demonstrated a cystic hemangioma In the wall of the cyst there were many cavernous, deformed structures The wall was covered by a thin layer of endothelium which apparently showed regional metaplasia In the spleen itself there were many hemorrhages and small infarctions especially close to the walls of the cyst (Dr S Falkmer)

The patient recovered She was discharged feeling well, on May 17

Discussion

A tumor discovered in late pregnancy or post partum will at first be interpreted as a complication to the delivery A diagnosis other than tumor seems more appropriate when the tumor is located below the left costal margin, suggesting splenomegaly

No sign or laboratory result supportive of a malignant disease e g acute leukemia, Hodgkin's disease or lymphosarcoma, was discovered Such a diagnosis was unlikely but could not be ruled out completely

A vascular lesion in the splenic vein or artery was instead proposed, in view of the rapid change in size of the tumor The appearance of chills and fever might initially suggest an inflammatory cause such as a splenic thrombophlebitis In those cases, however, a pelvic thrombophlebitis with secondary sepsis and embolization is usually present No primary thrombosis in the pelvic veins was discovered in this patient (10)

The diagnosis of a Banti's syndrome with a pre hepatic block was discussed but was abandoned in view of normal hematological data Even more uncommon diagnoses such as splenic vein rupture were considered (15)

Another and more likely diagnosis would be a ruptured aneurysm of the splenic artery, which is associated with splenomegaly in 44 % as shown by Owens and Coffey (16) Cases have been reported where marked splenomegaly has been caused by a subcapsular bleeding from such an aneurysm (13) It is peculiar that splenic artery aneurysm is twice as common in females as in males although all other aneurysms are overwhelmingly more prevalent in males by a ratio of 5 1 (23)

Arterial aneurysms tend to rupture during pregnancy About 50 % of all dissecting aortic aneurysms in women below the age of 40 occur during pregnancy (17) The reason is not known, they are thought to be secondary to either the increased blood volume during pregnancy, or endocrine factors or both Thus, Stuchlik (24) demonstrated more necrotic areas in the media of the arteries in a group of pregnant women compared with a group of non pregnant Others have instead explained the increased risk during pregnancy of rupture of splenic artery aneurysms as due to an elevated diaphragm in combination with a short splenic artery (12)

Most ruptured intra abdominal aneurysms have in common a two stage course

or double rupture Our patient had this two-stage development and a tentative diagnosis of splenic artery aneurysm, although the tumor was not pulsatile and no bruit was heard (6, 19, 20 22)

Menstruation and pregnancy have long been mentioned in the literature in the etiology of splenic cysts the spleen then becoming more congested (25) These cysts are, however, difficult to diagnose especially during pregnancy Duby (8) states that a downward displacement of the splenic flexure of the colon is practically pathogonomic of splenic cysts The value of this X ray finding is limited since it is not only cysts that can displace the colon flexure Also splenomegaly of different origin may cause the same displacement In our case this displacement indeed occurred The advent of celiac angiography will certainly increase our diagnostic possibilities in respect of splenic cysts (1)

The different splenic cysts have been classified by Duggan (9) as

I True cysts (lined by secreting membrane)
  A Epithelial (dermoid epidermoid)
  B Endothelial (lymphangioma, hemangioma polycystic disease)
  C Parasitic (lined by protoplasmic matrix containing numerous nuclei e g Hydatid cyst (caused by echinococcus)

II False cysts (no secreting lining)
  A Hemorrhagic
  B Serous
  C Inflammatory
    1 Acute necrosis from infection
    2 Chronic tuberculosis
  D Degenerating liquefaction of infarcted areas caused by embolism or arterial thrombosis

The true cysts are rare and often reach enormous size, requiring splenectomy The dermoid cysts may cause prominent splenomegaly and a spleen weight of more than 3 500 g has been reported The rarest of all splenic cysts is the epidermoid Only 13 cases have been reported in the literature (7)

True cysts may degenerate and become malignant but it is extremely rare

The angiomas are more common than the epithelial cysts but rarer than the false cysts (2, 3 11 14 18 21) Our patient was interesting in having had a course that classically is seen in rupture of an abdominal aneurysm One similar case has been found in the literature, Berger (4) reported in 1939 a two-stage rupture of a solitary splenic cyst The different false cysts are more common than the true cysts Trauma is an important etiological factor but spontaneous intrasplenic hemorrhages may also cause cyst formation

In diseases such as malaria kala azar syphilis, typhoid and mumps there is frequently a big fragile spleen which is susceptible to trauma Intrasplenic hemorrhages occur and gradually cysts will be produced

Symptoms are as a rule minimal unless the cysts are very large (5) There may be a sense of heaviness or a dragging kind of pain in the left upper abdomen Pressure on the gastro intestinal tract may lead to epigastric discomfort especially while eating The left leaf of the diaphragm is elevated and the lower left rib cage tends to flare outward Our

patient did not have any of the symptoms mentioned, although she was slightly asymmetric in the lower thorax in the manner described

With a two stage rupture of the spleen, splenic artery aneurysm, or splenic cyst, the prognosis is extremely poor if the diagnosis is not made in the free interval and operation performed The results published refer to rupture of splenic artery aneurysms and are discouraging Tomsykoski et al (26) reviewed 28 cases of splenic artery aneurysms rupturing during pregnancy Four died immediately Two of six survived after laparotomy All others died within six weeks of the onset of symptoms Only one living infant was obtained

Summary

A 23 year old female revealed a few days post partum, a growing tumor in the left upper quadrant of the abdomen The course was very similar to that of a two stage rupture with a possibility of either rupture of a splenic artery aneurysm or a splenic cyst An emergency operation was performed revealing an enlarged spleen with a prominent cyst The microscopic examination showed a cystic angioma Furthermore many hemorrhages and infarctions were demonstrated in the spleen A discussion is given of the problems of a splenic tumor appearing in late pregnancy or post partum

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Tyramine Test and Phaeochromocytoma

By

Wilfried von Studnitz and Otto Ljungberg

Advances in our knowledge of catecholamine metabolism have facilitated the diagnosis of phaeochromocytoma Chemical determination of catechol amines and their metabolites in the urine is now a routine procedure in many hospitals In the presence of a phaeochromocytoma the excretion of noradrenaline and adrenaline and their metabolites often fluctuates substantially The rate at which catecholamines are released from the tumour also seems to vary Owing to this inconstancy urin analysis is often unrewarding A series of other diagnostic tools, mainly pharmacological tests have therefore been developed These tests require measurement of the blood pressure after administration of an adrenolytic or sympatholytic drug (Piperoxane Regitine) or after administration of glucagon metacholine or histamine, for example to stimulate the release of catechol amines All these tests occasionally give false positive or false negative results More or less serious side reactions also limit the value of these tests Engelman and Sjoerdsma (1) developed a new provocative test the tyramine test for the diagnosis of phaeochromocytoma We have tried this test in four patients with a phaeochromocytoma known to be functional The results are given below

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Material and methods

The material consisted of four female in patients aged 44 to 56 in whom biochemical methods had given a firm diagnosis of phaeochromocytoma All four had a history of attacks of more or less pronounced tachycardia and of hypertensive crisis often combined with headache sweating pallor and sometimes a sensation of tingling in the fingers Three patients were normotensive while the fourth had been hypertensive for several years

The tyramine test was performed in the way described by Engelman and Sjoerdsma (1) Tyramine hydrochloride was obtained from Fluka (Buchs Switzerland) and from Hoffmann La Roche (Basel Switzerland) and the solution for injection was prepared as described by Engelman and Sjoerdsma (1) The patients received increasing doses of tyramine i v the largest single dose being

patient had a predominantly adrenaline secreting tumour This explanation is not supported by our study, for one of our patients (no 4) with a mainly noradrenaline secreting tumour did not respond to tyramine

Summary

The tyramine test described by Engelman and Sjoerdsma was used on four patients with phaeochromocytoma None of them reacted with an increase in the blood pressure In all of them there also was or had been some other endocrine tumour Three patients — belonging to the same family — had a medullary thyroid cancer and the fourth a functioning adenoma of the parathyroid gland In all four patients the excretion of 3 methoxy-4 hydroxymandelic acid or methoxycatecholamines was increased In the three related patients the urinary excretion of metadrenaline represented at least 50 % of the total methoxycatecholamines

Acknowledgement

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Testing of Exocrine Function of Pancreas in Diabetes Mellitus by Use of ^{75}Se-methionine and of Secretin

By

JUHANI LAHDEVIRTA

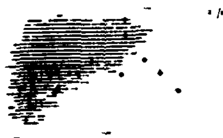
There are reports that diabetes mellitus is sometimes associated with an impaired exocrine function of the pancreas Jones et al (9) have found in 50 % of diabetics a lowered activity of the pancreatic enzymes in the duodenum Ågren et al (18) found in half of their diabetics an abnormal result in the secretin test as did Pollard et al (11) in eight out of 13 diabetics and Dreiling (6) in 27 out of 62 diabetics Chey et al (4) observed an abnormal exocrine function of the pancreas in 18 out of 48 diabetics Vacca et al (13) stated that in 73 % of diabetics the secretin test had given a low value In most cases the exocrine pancreatic insufficiency is asymptomatic

Isotopic scanning of the pancreas with Se methionine was introduced by Blau et al in 1962 (2 3) The pancreas actively produces digestive enzymes and takes up this amino acid sufficiently to allow of measurement with present scanning devices The uptake per unit weight is greatest in the pancreas it is two to ten times that of the liver The liver however leads in total uptake (17) and this may upset the estimation of the pancreatic uptake where the organs overlap

In 1962 Blau and Bender (2) stated that they had been able to visualize the pancreatic uptake in about two thirds of patients without pancreatic disease Haynie et al (8) discerned the uptake in four of five of their patients (80 %) Other series including also pathological cases have given the following figures Blau (1) 85 % Sodee (12) 97 % Lahdevirta and Haikonen (10) 84 % and Diethelm and Haacke (5) 68 % A pancreatic tumour impairs the uptake chronic pancreatitis reduces it throughout the organ and acute pancreatitis abolishes the uptake

It has now been sought to study the pancreatic uptake of selenomethionine in diabetics and to compare this method with duodenal analysis after secretin stimulation

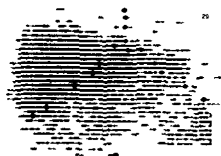
Submitted for publication March 13 1967



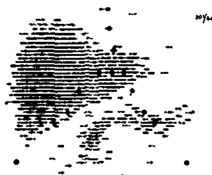
F g 1 Zero-uptake n the pancreas No def n e uptake demonstrable



F g ? The pancreat c uptake of selenometh o n ne scored as a one po nt uptake



F g 3 The pancreat c uptake of selenometh o n ne scored as a two po nts up ake



F g 4 The pancreat c uptake of selenometh o n ne scored as a three po nts uptake

Materıal

The cases stud ed v ere 27 hosp talızed d a bet cs At the t me of exam nat on their d a bete as n balance None hade cl n cal s gns of chron c pan reat t s or malabsopt on or per ods of d arrhoea The group of non ınsul n d abetics cons s ed of 15 pat ents ten females and five males mean age 6? years (from 46 to 71 years) Four of them v ere treated onl th d et Nine v ere obese The dura on of their man fest d a be es varied from one month to ten year

The group of nsul n dependent d abetics consis ed of twel e pa ents three females and n ne males mean age 45 years (from 23 to 68 years) F e v ere obese and six v ere ju en le d abet cs The durat on of the r man fest d abetes var ed from t months to 32 years

The control group ıncluded 28 non d a bet c pat ents ho v ere hosp talized for various non pancreat c d seases They had no glu osur a and the r fasting blood sugar as normal They had neither d arrhoea nor s gns of malab orpt on Th re were 11 females and 1 male th a mean age of 43 years (from 32 to 76 years) Th rteen of them v ere obese

Methods

The selenometh onıne scann ng

The [7] Se L-methıon ne came from the Br t sl Rad ochem cal Centre The dose g en was 4 μC /kg body v e ght After an overn ght

fast two to three glasses of milk were given at 8 a m 135 units of pancreozymin (Boots Pure Drug Co Ltd Nottingham) were administered 1 1/2 to 2 hours after the ingestion of the milk Selenomethionine was administered 1 to 2 hours later and scanning was commenced after 30 min The scanning device was a Magnascanner II manufactured by Picker X ray Corp Waite Mfg Div Inc Cleveland Ohio with a 3 inch crystal and coarse focus collimator with 19 holes During the scanning the patient was lying on his back on the examination table with his feet and left flank slightly raised The epigastric angle was marked on the picture with large black dots

In order to compare the scanning results with one another the degree of uptake was scored from zero to three as in the previous paper (10) The uptake is zero when no definite uptake can be established (fig 1) An uptake of one point is weak but detectable (fig 2) An uptake of three points is high and enables the shape of the pancreas to be discerned (fig 4) An uptake of two points is intermediate between one and three (fig 3)

If the uptake by the liver interfered with estimation of the pancreatic uptake a liver scanning with colloidal cold (^{198} Au 150 μCi) was carried out to differentiate them In four cases the liver extended so far down that it was not possible to estimate the uptake of the pancreas These cases have been excluded from this material

The secretin test

In 19 cases the secretin test was performed according to Dreiling and Janowitz (7) Secretin (Boots Pure Drug Co Ltd Nottingham) was given intravenously one unit/kg body weight During 80 min six samples of duodenal juice were aspirated and from these the highest bicarbonate content and the total volume were estimated A total volume of at least 2 1 ml/kg body weight in 80 min was considered as normal bicarbonate content between 75 and 89 mEq/l as equivocal and below 75 mEq l as a clearly pathological result indicating a pancreatic exocrine insufficiency

Results

Control group

Fig 5 shows the pancreatic uptake values in the control group in relation to age There were few cases in the older age groups, but it seems that the uptake decreases slightly with age The patients below 50 years have a mean uptake value of 2 5 and those over 50 a value of 2 07

Table I gives the distribution of cases according to uptake values In the control group a detectable pancreatic uptake of selenomethionine took place in 96 5 % in agreement with Sodee (12) An uptake of at least two points was present in 89 5 % The mean value for the uptake in this group was 2 3

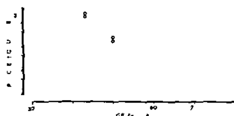


Fig 5 Correlation between pancreatic uptake and age in control group

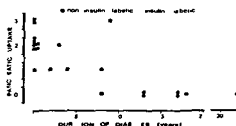
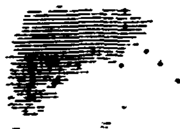


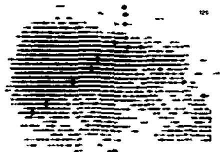
Fig 6 Correlation between pancreatic uptake and duration of the diabetes



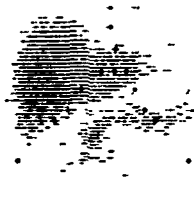
F g 1 Zero-uptake in the pancreas No def n e uptake demonstrable



F g 2 The pancreat c uptake of selenometh o-n ne scored a a one po nt uptake



F g 3 The pancrea c uptake of selenometh o n ne scored as a two-po nts uptake



F g 4 The pancreat c uptake of selenometh o n ne scored as a three po nts uptake

Material

The cases s ud ed ere 2 ho p alized d a bet cs At the t me of exam nat on their d a bete was n balance None hade cl n al s gns of chron pancreat s or malabsop on. or per ods of d arrhoea The group of non in ul n d abe cs cons ed of 15 pa en s ten females and f e mal s mean age 62 years from 46 o 1 years Four of them e e treated onl h d et Nine ere obe e The dura on of the r man fest d a be es var ed from one month to en years

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Methods

The elenometh on ne scann ng

The Se L-meth on ne came from the B sl Rad ochem cal Centre The dose g en as 4 μC /kg body e ght After an o ern ght

TABLE II Correlation between pancreatic uptake and secretin test

Pancreatic uptake 0—3 points	No of cases	Secretin test				
		Volume		Bicarbonate		
		Normal >2 1 (ml/kg/80 min)	Low <2 1 (ml/kg/80 min)	Normal >90 (mEq/l)	Equivocal 75—89 (mEq/l)	Low <75 (mEq/l)
Controls						
3	5	3	2	5		
2	3	2	1		3	
1	1	1			1	
0	0					
Diabetics						
3	1	1			1	
2	3	3		1	2	
1	1		1		1	
0	5	1	4			5

of a digital output There are several factors which diminish the accuracy of the scanning method as a measure of true pancreatic uptake thus there is normally some variability in the size and shape of the pancreas The sensitivity of the scanning device may vary in spite of checking especially when examinations are made over a long period or with different devices A thick abdominal wall diminishes the number of observed counts both through increased absorption and through excessive distance from the collimation focus The amount of food eaten in the days preceding the examination may vary starvation can diminish the pancreatic uptake (1 10)

The results suggest that the pancreatic uptake decreases in insulin dependent diabetes as a function of the duration of the disease If the degree of uptake is considered to reflect exocrine function the results agree well with those of Vacca et al (13) On the other hand Chey et al (4) using the pancreozymin secretin test could not find any correlation between pancreatic secretion duration of disease severity of disease and the amount of insulin needed In the present work the lowest mean pancreatic uptake was found in juvenile diabetics which agrees with the observation of Chey et al (4) Obese insulin dependent diabetics had a better uptake than non obese which agrees with the view of Vacca et al (13) that the pancreatic exocrine function is better in obese diabetics In the group of non insulin diabetics however, non-obese diabetics had better uptake than obese In this group the duration of the diabetes did not have a clear influence

Comparison of the pancreatic scanning with the secretin test (table II)

indicates that both in the control group and in diabetics the degree of uptake matches the results of the secretin test Since in the secretin test only the volume and the highest bicarbonate content were considered, pancreatic scanning and the secretin test measure different things Secretin increases the volume and bicarbonate content of the pancreatic juice, while the scanning method used measures the incorporation of selenomethionine into enzyme proteins (14) However, the lowered exocrine function of the pancreas as estimated by the secretin test probably has the same causation as that reflected in the decreased uptake of selenomethionine viz pancreatic fibrosis, fatty infiltration or lipomatosis as found in some diabetics (15 16)

From the pancreatic uptake of ^{75}Se methionine one can evidently evaluate at least semi quantitatively the exocrine function of the pancreas The pancreatic uptake is not manifest in all so called normal cases which may be due to inclusion of cases with asymptomatic pancreatic insufficiency From the material selected it indeed seems feasible to visualize the pancreas in 97 % of cases as Sodee (12) shows If the pancreas is not revealed by scanning, investigating of its exocrine function with the secretin test is warranted In the cases with no clinical symptoms of pancreatic exocrine insufficiency a low uptake found by scanning does not always mean a technical failure

Summary

The pancreatic uptake of ^{75}Se methionine in 27 diabetics who had no symptoms of chronic pancreatitis was studied by external scanning The uptake shown by scanning was graded in four categories and the comparison of results was based on this scale The zero point and one point uptakes were considered as pathological the three points uptake as normal, and the two points uptake in most cases as normal In the control group there was at least a two points uptake in 89 5 % In the 15 non insulin diabetics the corresponding percentile was 66 5 and in the 12 insulin dependent diabetics 16 5 Nineteen patients were studied by means of the secretin test, and the results were largely in agreement with the pancreatic uptake of ^{75}Se methionine

Conclusions ^{75}Se methionine scanning gives a rough measure of pancreatic exocrine function, which is deficient in many insulin dependent diabetics

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From the Division of Clinical Endocrinology (Head H Doorenbos M D) of the Department of Internal Medicine (Head E Mandema M D), State University Groningen the Netherlands

The Relationship Between Serum Free Fatty Acids and Blood Sugar in Non-obese and Obese Diabetics

By

W D Reitsma

The energy supply of the human body is largely derived from free fatty acids (FFA) and glucose During prolonged fasting FFA are the predominant fuel Glucose is mainly used to meet oxidative demands just after eating

Most tissues are able to use both glucose and FFA as sources of energy In nerve cells and erythrocytes however only glucose is utilized The levels of serum FFA and blood sugar depend on the metabolic processes in adipose tissue muscles and liver

Circulating FFA are bound to plasma albumin They represent the major transport form of fat from adipose tissue to the sites of utilization The turn over rate of this fat fraction in blood is about 30 % min In situations where glucose utilization is not possible as in normal persons during starvation the serum level of FFA rises markedly After glucose ingestion the FFA concentration falls (2)

Randle et al (11) have clarified the relationship between glucose and fatty acid metabolism for which they introduced the term 'glucose fatty acid cycle According to them insulin enhances the entry of glucose into adipose tissue if glucose is available This leads to the formation of alpha glycerophosphate which is used for the esterification of FFA to form triglycerides As a result the output of FFA from adipose tissue diminishes and the serum concentration of FFA falls to remain low as long as glucose is available

During fasting the FFA content of plasma rises because of lipolysis in adipose tissue An elevated plasma FFA level inhibits he utilization of glucose by muscle, which is reflected in a decrease of insulin sensitivity This mechanism helps to maintain a constant plasma glucose concentration during starvation

Several hormones influence FFA and glucose metabolism Insulin lowers the

Submitted for publication March 13 1967

concentration of both glucose and FFA in blood (2) Growth hormone accelerates FFA release from adipose tissue, markedly raises blood FFA (9), and it causes a secondary rise in blood sugar by inhibiting glucose utilization in muscle

In this study the effect of fasting and of glucose ingestion on serum FFA concentrations in obese and in non obese diabetics was investigated In patients with diabetic (pre)coma the effect of insulin therapy on glucose and FFA concentrations was studied In some patients the plasma insulin and growth hormone concentrations were also measured

Material and methods

Blood sugar concentrations were estimated according to Somogyi Nelson on venous blood (15) Serum FFA were measured by Dole s titration procedure (2) Plasma insulin was estimated by the radio immunological technique of Yalow and Berson (17) Plasma growth hormone was determined radio-immunologically Iodination was accomplished by the Greenwood et al procedure (4) The iodinated product was purified by dialysis and by Sephadex G 200 filtration separation of free and antibody bound growth hormone was accomplished after an incubation period of four days by a hydrodynamic flow technique on Whatman 3 M C paper according to Touber (16) All the obese patients were

TABLE I Fasting FFA levels (μEq/l) in normal controls obese persons obese diabetics non obese diabetics and patients with diabetic (pre)coma

Normal	Obese	Obese diabetic	Non obese diabetic	Diabetic (pre)coma
400	366	407	666	984
431	407	416	721	1138
438	418	495	746	1244
440	463	524	802	1326
456	644	539	861	1453
489	647	592	863	1898
510	674	681	938	2094
551	728	735	950	2119
554	822	746	991	2296
559	920	781	1277	3252
593	1087	800		3306
614	1180	833		
672	1263	840		
700		994		
731		1026		
731		1047		
827		1067		
		1122		
		1140		
		1147		
		1487		

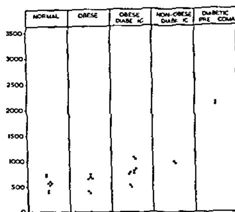


Fig 1 Fasting FFA levels μEq/l in normal controls obese persons obese diabetics non obese diabetics and patients with diabetic (pre)coma

more than 10% over weight The body weight of the normal subjects serving as controls and of the non-obese diabetics was between 80 and 100% of normal body weight i e their body weight expressed in kg was between 80 and 100% of their height in cm above one m Age and sex distributions were the same in all groups The diabetics had either spontaneously elevated blood sugar values of more than 180 mg% or a glucose tolerance test with a maximum blood sugar value of more than 180 mg% They did not receive treatment before the clinical study started Only the patients in diabetic (pre)coma and the patients in whom growth hormone studies were done had previously been treated with insulin

Results

FFA and blood sugar concentrations were investigated in the fasting state, during a continued fast as well as after glucose ingestion Fasting FFA levels found in normal subjects apparently healthy obese persons obese diabetics non obese diabetics and patients with diabetic (pre)coma were compared The data are presented in fig 1 and table I

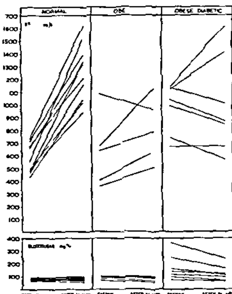


Fig 2 The effect of 24 hours fasting on levels of serum FFA and blood sugar in normal individuals obese subjects and obese diabetics

It is concluded that the fasting level of FFA in obese diabetics is higher than in normal persons (Wilcoxon test $P < 0.05$) No such difference in FFA levels was observed between obese people and normal subjects However there is a tendency for the FFA level to be abnormally high in obese people and particularly in obese diabetics (*combined Wilcoxon test* $P < 0.05$) Also the non obese diabetics show a higher FFA level than the normal subjects (Wilcoxon test $P < 0.01$) Very high FFA levels are found in patients with diabetic (pre)coma

The changes in serum FFA and blood sugar concentrations during a 24 hour fast were investigated in normals obese persons and obese diabetics (fig 2) Between normal and obese persons there is a distinct difference in the behaviour of

TABLE II The effect of oral administration of 50 g glucose on levels of plasma insulin serum FFA and blood sugar in normal individuals, obese diabetics and non obese diabetics

	Insulin (μU/ml)			FFA (μEq/l)			Blood sugar (mg %)		
	Fasting	After 1 hr	After 2 hrs	Fasting	After 1 hr	After 2 hrs	Fasting	After 1 hr	After 2 hrs
Normal controls	2	27	7	510	258	317	64	90	49
	19	56	16	440	387	327	84	82	67
	3	23	5	551	272	300	89	108	68
	18	42	14	554	248	313	91	98	73
	20	45	19	827	280	407	78	78	75
	8	49	32	700	347	353	87	142	97
Obese diabetics	19	35	41	407	571	311	139	257	180
	10	8	7	833	566	1007	362	645	600
	9	38	28	800	351	400	149	270	160
	22	>200	35	840	153	147	122	186	86
	44	72	108	1140	1290	794	240	444	431
	24	83	112	1067	700	367	106	259	254
Non obese diabetics	0	10	2	721	669	871	276	432	412
	1	10	8	746	933	1020	283	426	430
	5	10	12	1277	1153	840	266	440	493

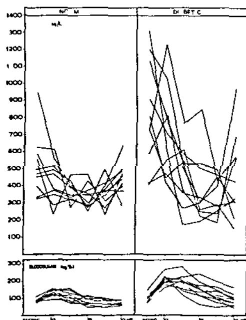


Fig 3 Levels of serum FFA and blood sugar in healthy individuals and obese diabetics during a glucose tolerance test (50 g orally)

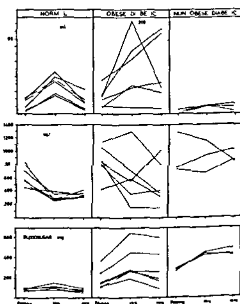


Fig 4 The effect of oral administration of 50 g glucose on levels of plasma insulin serum FFA and blood sugar in normal individuals, obese diabetics and non-obese diabetics

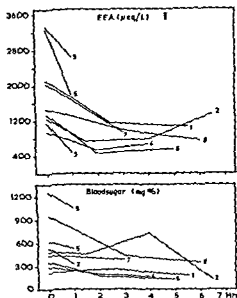


Fig 5 The effect of therapy with regular insulin on levels of serum FFA and blood sugar in patients with diabetic (pre)coma

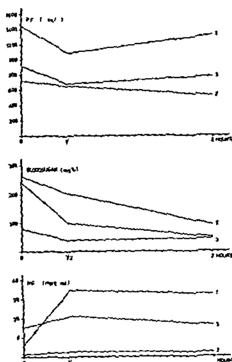


Fig 6 The effect of the intravenous administration of 40 U regular insulin on levels of serum FFA blood sugar and plasma growth hormone in three patients with juvenile type of diabetes

serum FFA The rise of FFA after a 24 hour fast is smaller in the obese than in the normal subjects (Wilcoxon test P< 0 01) In the obese diabetics blood sugar falls to normal or nearly normal levels during such a prolonged fast

The effect of oral administration of 50 g glucose on FFA level and blood sugar concentration was measured in normal persons and obese diabetics The results are presented in fig 3 In both groups FFA fall precipitously In the control subjects the blood sugar remains within the normal range in the obese diabetics marked hyperglycemia is found The lowest FFA level in the obese diabetics was reached at a later moment than in normals (combined Wilcoxon test P<0 01) This retarded fall of the FFA levels is in agreement with the retarded but excessive insulin response known to occur after glucose loading in diabetes of early maturity onset (18)

In fig 4 and table II the effect of oral administration of 50 g glucose on plasma insulin concentration serum FFA levels and blood sugar concentration is shown for these three groups of persons In the normal individuals there is after one hour a rise of the insulin concentration as compared with the fasting value after two hours the insulin concentration returns to about the fasting level As insulin is known to lower the FFA concentration in the blood the rapid fall of the FFA level after glucose ingestion accords with the

immediate insulin response known to occur in normal subjects In the obese diabetics there also is an insulin response after glucose ingestion but no consistent difference is found between the one hour and the two hour concentrations In three patients the insulin response is excessive, while in one patient there is no rise in the insulin concentration Only in the latter patient does the FFA concentration not fall below the fasting level after two hours In the three non obese diabetics the insulin response is negligible and in only one of them does the FFA concentration fall It would appear that in the one obese diabetic in whom there was no rise of insulin concentration after glucose ingestion, insulin and FFA behave in the same manner as in non-obese diabetics

The effect of therapy with high doses of regular insulin on blood sugar con centration and serum FFA concentra tion was investigated in nine patients with diabetic (pre)coma The results are presented in fig 5 where for each patient the blood sugar and FFA levels are indicated by the same number It will be seen that during insulin treat ment FFA and blood sugar fall con comitantly

In three patients who needed high doses of insulin the effect of one dose of 40 U regular insulin on the concen tration of blood sugar and serum growth hormone was investigated The data are presented in fig 6 again for each pa tient the concentrations of FFA blood sugar and growth hormone are indicated by the same number All three patients show a marked fall of the blood sugar level In two patients there is a rise of the growth hormone concentration in the first half hour after insulin ad ministration, in these patients the FFA level falls initially, but later rises again In the third patient there is no rise in growth hormone level, nor is there a secondary rise in the level of FFA

Discussion

The results obtained in obese persons and obese diabetics will be discussed to gether Serum FFA of both the obese in apparent good health and the obese dia betic do not rise during prolonged fast ing As the serum FFA level depends on the equilibrium between FFA mobili zation and utilization the smaller FFA rise during fasting can be explained either by diminished lipolysis from adi pose tissue or by increased utilization Gordon et al (3) who injected albumin C^{14} palmitate intravenously in normals and in obese people and measured $C^{14}O_2$ production, did not find a higher $C^{14}O_2$ production in the obese Apparently the net mobilization of FFA is diminished during fasting in obese patients

Growth hormone is of importance for the mobilization of FFA from adipose tissue during starvation Patients with panhypopituitarism on substitution therapy with cortisol and thyroid pow der show a smaller rise in serum FFA during 24 hours fasting than do normal subjects Injection of growth hormone repairs the metabolic abnormality in these patients (12) In normal persons growth hormone levels rise appreciably during fasting to fall again after the intake of glucose (6, 13) It was found

by Beck et al (1) that in obese individuals the plasma growth hormone level remains low throughout starvation for several days This holds true both for obese persons with a normal glucose tolerance prior to fasting and for obese diabetics The fact that the level of serum growth hormone does not rise seems to explain the relative inability of obese patients to mobilize FFA during a prolonged fast In this respect they behave like patients with panhypopituitarism

The fall of the blood sugar levels during prolonged fasting in obese diabetics may be caused by their residual insulin The excessive insulin response after a glucose load in obese diabetics was first reported by Yalow and Berson (18) They found that although the rise of the insulin concentration was retarded it was higher and more sustained than in normal subjects These high insulin levels can lead to reactive hypoglycemia This phenomenon has long been recognized as an early symptom in obese diabetics (14) The retarded rise of insulin is in agreement with the retarded fall of the FFA levels shown in fig 3

Karam et al (7) found that obese persons without demonstrable impairment of glucose tolerance also show an excessive insulin response to glucose loading but without a delay The reactive hyperinsulinism in obese persons and obese diabetics is reduced after pretreatment with 150 mg DBI (phenformin) daily for three days (5) Though there is a smaller rise of the insulin level after phenformin there also is less hyperglycemia DBI causes a fall of the blood sugar level probably by increasing peripheral glucose uptake through stimulation of anaerobic glycolysis The explanation of the reduced insulin response to glucose after DBI pretreatment seems to be that the stimulation of the pancreas is less because cellular glucose utilization is facilitated

A peripheral resistance to insulin in obese patients explains the finding of a normal glucose tolerance in the presence of an abnormally large increase in circulating insulin Hyperinsulinism is then to be considered a compensatory reaction The investigations of Rabinowitz and Zierler (10) on forearm metabolism give some support to this concept In obese subjects they found less increase of glucose uptake after administration of insulin than in normals Until now no differences have been found between obese persons with and without diabetes as regards the behaviour of serum FFA growth hormone and insulin during fasting and after the ingestion of glucose

A lack of rise in insulin concentration after glucose ingestion as observed in one obese diabetic, has earlier been reported by Maingay (8) In these cases the insulin reserve is insufficient and carbohydrate metabolism is severely disturbed There is no distinct fall in FFA after glucose ingestion This could well be due to exhaustion of β cells of the pancreas through prolonged hyperstimulation in the preceding years These cases approach the condition of absent insulin reserve observed in juvenile diabetics and one may expect them soon to lose weight A change to the juvenile type of diabetes is known to occur suddenly in some obese diabetics

In patients with diabetic (pre)coma

the high FFA levels result from the fact that glucose cannot be utilized Under these circumstances a high growth hormone concentration is to be expected The high FFA levels further inhibit glucose utilization by muscle tissue This explains why there is a marked insulin insensitivity in this situation and why high doses of insulin are needed to lower the blood sugar concentration If the blood sugar concentration falls, FFA levels fall concomitantly

Hypoglycemia is also a potent stimulus for growth hormone secretion in normals (13) In diabetic persons on insulin therapy a sudden fall in the blood-sugar concentration coincides with a rise in the plasma growth hormone level and a stimulation of the secretion of epinephrine

This may occur before hypoglycemic values are reached and can lead to a secondary rise of the FFA levels In diabetic patients on insulin therapy continuous changes in blood sugar concentrations FFA levels and growth hormone concentrations are thus to be expected This may explain why fasting FFA levels are elevated in non-obese diabetics and why the height of the FFA level in these persons in the fasting state is not directly related to the blood sugar concentration

Summary

1 The behaviour of FFA in obese diabetics differs from that in normal subjects Fasting FFA levels are higher the rise during prolonged starvation is smaller and the fall after glucose ingestion is retarded

2 Glucose ingestion leads to an excessive insulin response in the majority of obese diabetics the rise of the plasma insulin concentration being prolonged in these patients This explains the retarded fall of FFA In non obese diabetics the insulin response after glucose ingestion is very small This type of insulin response is occasionally also found in obese diabetics

3 During treatment of diabetic (pre) coma with regular insulin the levels of FFA and of blood sugar fall concomitantly

4 The results obtained in juvenile diabetics can be interpreted with the following hypothesis A sudden fall of the blood sugar concentration after insulin administration stimulates growth hormone secretion This leads to a rise of the FFA levels and explains why the blood sugar level and the FFA concentration do not rise and fall concomitantly in this type of diabetes

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4 The results obtained in juvenile diabetics can be interpreted with the following hypothesis A sudden fall of the blood sugar concentration after insulin administration stimulates growth hormone secretion This leads to a rise of the FFA levels and explains why the blood sugar level and the FFA concentration do not rise and fall concomitantly in this type of diabetes

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From the Department of Internal Medicine (Head N Soderstrom, M D) University of Lund, Lund, Sweden

Studies on Fatty Acid Metabolism in Diabetics During Exercise

V Plasma concentration of free fatty acids and glycerol in newly diagnosed, adult diabetics during exercise

By

SVEN CARLSTROM

It was shown in earlier reports (5 7, 8) from this laboratory that when juvenile, male newly diagnosed diabetics performed a short period of exercise on a bicycle ergometer, they differed from control subjects of the same age in responding to the exercise with a more pronounced increase of fatty acid mobilization Thus, the plasma concentrations both of free fatty acid (FFA) and of glycerol rose to significantly higher levels in the diabetics than in the controls (5, 7 8) All diabetics in these studies were newly diagnosed diabetics of the juvenile type in whom the onset of disease occurred before the age of 35 years

In the present study diabetics with onset of disease after the age of 35 years have been examined and compared with control subjects of the same age to ascertain whether the same difference exists

Submitted for publication March 14 1967

Material and methods

The diabetic subjects were divided into two groups Group A contained newly diagnosed male diabetics with onset of disease between the ages of 35 and 45 and group B contained newly diagnosed male diabetics with onset of diabetes after the age of 45 In group A six diabetic subjects (cases D1—D6) and in group B five diabetic subjects (cases D7—D11) were studied Details concerning age weight height and some clinical data are given in table I

As controls for group A six apparently healthy subjects (cases C1—C6) between the ages of 35 and 45 were examined Similarly two apparently healthy men (cases C7—C8) above the age of 45 were examined as controls for group B Age height and weight of the control subjects are given in table II The control subjects had no family history of diabetes no glycosuria and normal fasting blood glucose concentrations

The examination started in the morning with the insertion of one arterial and one venous catheter the subjects were then al

TABLE I. Some clinical data for the diabetic subjects examined

Group	Case	Age (yrs)	Height/weight (cm/kg)	Plasma creatinine (mg/100 ml)	Duration of symptoms (weeks)	Complications	Treatment after the experiment
A	D1	41	173/71.0	0.9	52 ?	0	Diet
	D2	39	180/71.5	0.8	4	0	Diet+insulin
	D3	43	182/68.0	0.8	5	0	Diet+insulin
	D4	44	170/58.7	1.0	8	0	Diet+chlorpropamide
	D5	43	174/75.5	0.8	—	0	Diet+insulin
	D6	37	172/88.5	—	2	0	Diet+insulin
B	D7	46	177/63.0	1.0	3	0	Diet+insulin
	D8	59	167/56.0	1.0	10	0	Diet+chlorpropamide
	D9	69	171/73.5	NPN 28	6	?	Diet+tolbutamide
	D10	47	172/69.4	—	4	0	Diet+chlorpropamide
	D11	61	181/80.0	1.0	6	0	Diet+tolbutamide

NPN = non protein nitrogen (mg/100 ml)

TABLE II. Some data for the control subjects

Case	Age (yrs)	Height/weight (cm/kg)
C1	37	176/75.5
C2	43	168/64.0
C3	35	169/62.5
C4	35	172/75.0
C5	43	174/73.0
C6	41	179/82.0
C7	48	163/60.0
C8	49	175/87.0

lowed to rest for 1 1/2 hours. After rest the subjects performed graded submaximal exercise on a bicycle ergometer loaded with either 300 or 600 kpm/min, the load was chosen with regard to the physical working capacity of the subject examined. For diabetics cases D8, D9 and D10 the load was only 300 kpm/min. For all other subjects examined the load was 600 kpm/min. After the exercise period all subjects were allowed *to rest for 1—1 1/2 hours. During the entire* experiment blood samples were withdrawn through the arterial catheter for determinations of plasma FFA, blood glucose and, in most cases, plasma glycerol concentrations. ECG, heart rate and intra-arterial pressures were recorded throughout.

Cardiac output, respiratory quotient (RQ), pH and base excess were estimated at rest and during exercise. Details of the techniques used for hemodynamic and chemical measurements have been published earlier (5, 6).

Patients D4 and D8 had been given a light breakfast about two hours prior to the examination. The other subjects were examined after an overnight fast. In patients D4 and D8 a slightly heparinized (50 mg/500 ml) NaCl solution was used to avoid blood clotting in the catheters. About 25 ml of this solution (i.e. about 2.5 mg of heparin) was given during the entire experimental period. Later it was found that addition of heparin was not imperative and all other subjects were examined without addition of heparin.

After the examination the diabetics were *found to require various forms of treatment* for control of the disease. In table I the treatment of each patient is given.

The statistical evaluation was made mostly according to Wilcoxon s rank sum test (17) However when the number of subjects was less than four (as in one of the groups compared) it was necessary to use the Student s t test (9)

Results

The plasma FFA concentrations in groups A and B are given in tables III and IV respectively The plasma FFA concentrations in the control subjects are given in tables V and VI All values are summarized in figs 1 and 2 It is evident from the data that the mean plasma FFA concentrations in group A at rest exceeded those in the controls of the same age (cases C1 —C6)

The significance levels are given in table III This difference was maintained during exercise and increased further during the period up to 25 min after the beginning of exercise The plasma FFA concentrations in the diabetics then decreased and after 55 min there was no significant difference from the controls

The mean plasma FFA values for group B did not differ significantly from those for the two controls of the same age (cases C7 and C8) at any time during the experiment

The plasma glycerol concentrations in groups A and B are given in tables VII and VIII respectively The same data for the controls corresponding to groups A and B are given in tables IX and X All plasma glycerol concentrations are graphically summarized in figs 1 and 2

At one point during the rest prior to exercise the mean plasma glycerol concentrations in group A were significantly higher than in the controls In up to 40 min from the beginning of exercise the mean plasma-glycerol concentrations in group A were higher than those in the controls of the same age (cases D1—D6) The significance levels are given in table VII

The mean values of the plasma glycerol concentrations in group B tended to be higher than those in the controls of the same age (cases C7—C8) but statistical significance is reached only on some occasions This is possibly due to the low number of control subjects The significance levels are given in table VIII

The mean glycerol concentrations after exercise have been considered further The lowest estimated mean glycerol concentration in the group A subjects in the period after exercise has been subtracted from the mean values for group A These mean increment concentrations have been plotted on semilogarithmic paper against time The points fall roughly on a straight line Also at each point of time after exercise the mean increment glycerol concentrations for the other three groups have been calculated and plotted on the same diagram (fig 3) For each group, the points fall roughly on a straight line Furthermore, the lines for the four groups show approximately the same slope

The blood-glucose concentrations in group A group B and the control subjects are summarized in table XI It is evident that the mean blood glucose concentrations are higher in the diabetic groups than in the corresponding groups of controls

During the experiment no significant

TABLE III Plasma FFA concentrations during the experiment (mEq/l) The significance levels in controls C1 — C6 (table V) are given on the bottom line of the table

Case	Time										
	Hours				Minutes						
	—2	—1 1/2	—1	—1/2	1	3	5	8	11	13	15
D1	0.70	0.52	0.61	0.72	0.83	0.74	0.80	0.86	1.03	—	0.99
D2	0.44	0.92	0.95	0.88	1.04	0.82	0.72	0.58	0.77	1.16	1.19
D3	0.92	0.71	0.86	1.17	1.36	1.00	0.88	1.22	1.12	1.67	1.82
D4	—	—	1.18	1.08	—	1.26	1.46	1.83	1.88	1.83	2.24
D5	0.98	0.52	0.84	1.87	1.78	1.62	1.40	1.68	2.25	2.46	2.88
D6	0.85	0.75	0.73	0.92	0.97	0.84	0.78	0.78	0.67	0.95	1.10
Mean	0.78	0.68	0.86	1.11	1.20	1.05	1.01	1.16	1.29	1.61	1.70
S.E.M	0.10	0.08	0.08	0.17	0.17	0.14	0.14	0.21	0.26	0.27	0.31
In comparison with C1—C6 p<	0.05	0.02	0.02	0.01	0.01	0.05	0.02	0.01	0.01	0.01	0.01

TABLE IV Plasma FFA concentrations during the experiment (mEq/l) No significant differences data for the controls C7—C8 (table VI)

Case	Time										
	Hours				Minutes						
	—2	—1 1/2	—1	—1/2	1	3	5	8	11	13	15
D7	0.64	0.52	0.53	0.67	0.79	0.77	0.74	0.94	1.41	—	—
D8	1.20	0.60	1.07	0.92	0.72	0.57	0.57	—	1.04	—	1.12
D9	0.63	0.56	0.63	0.56	0.83	0.73	0.71	0.84	1.09	1.10	1.14
D10	—	0.80	0.84	0.92	0.81	0.62	0.53	0.56	0.70	0.96	1.15
D11	0.64	0.66	0.73	0.84	0.87	0.66	0.53	0.54	0.64	0.94	1.06
Mean	0.78	0.63	0.76	0.78	0.80	0.67	0.62	0.72	0.98	1.00	1.12
S.E.M	0.14	0.05	0.09	0.07	0.03	0.04	0.05	0.10	0.14	0.05	0.02
In comparison with C7—C8	No significant differences										

differences were found between group A and the controls C1—C6 or between group B and the controls C7—C8, as regards heart rate, cardiac output, stroke volume and intra arterial pressures Nor were there any significant differences in oxygen uptake, pH, base excess RQ or hematocrit value between group A and

comparison with the corresponding data for the

18	25	40	55	70	100
1.04	1.03	0.65	0.53	0.51	0.46
1.42	1.46	1.30	1.08	0.89	1.01
2.01	2.10	1.64	1.15	1.08	1.42
2.51	2.25	—	—	—	—
3.48	3.53	2.72	1.93	1.47	1.02
1.16	1.18	1.36	0.95	0.97	0.94
1.94	1.93	1.53	1.13	0.98	0.98
0.38	0.38	0.34	0.23	0.15	0.15
0.01	0.01	0.05	—	—	—

were found in comparison with the corresponding

18	25	40	55	70	100
2.00	1.86	1.04	0.59	0.59	0.75
0.82	0.72	0.47	0.62	0.60	0.92
1.25	1.30	1.07	0.80	0.63	0.66
1.30	1.34	1.03	0.75	0.70	—
1.15	1.40	1.25	1.05	0.67	0.87
1.30	1.32	0.97	0.76	0.64	0.80
0.19	0.18	0.13	0.08	0.02	0.06

the controls C1 C6 or between group B and the controls C7 C8 The RQ values for groups A and B and the controls are given in table XII

Discussion

The plasma FFA concentrations both at rest and during exercise in the controls accord with earlier findings (2, 3, 4, 10, 15) The controls aged over 45 do not differ from the younger controls in the *earlier study (5) or the present one This* may excuse the fact that only two control subjects above 45 years of age were examined The plasma FFA concentrations at rest in the diabetics with onset of disease between the ages of 35 and 45 are higher than those in the controls of similar age This finding accords with earlier studies by many authors (1, 13 14) with findings from newly diagnosed younger diabetics from this laboratory (5) During the rest period prior to exercise there is a rise of the plasma FFA level *among the diabetics which is presumably* due to lipid mobilization initiated by fasting

The mean plasma-glycerol concentrations in group A subjects during the resting period tend to be higher than those in the controls, the difference being significant on one occasion There is also a tendency for the plasma glycerol concentrations to rise during the rest period which supports the theory of an increased lipid mobilization during the rest period Plasma-glycerol concentrations in newly diagnosed diabetics at rest have been estimated by Hales et al (11) who found higher mean fasting concentrations than in controls but the difference was not significant

The plasma FFA levels at rest in the diabetic group B are no higher than those in the controls of *similar age* The plasma-glycerol concentrations in the diabetics at rest may show a tendency to

Table V Plasma FFA concentrations during the experiment (mEq/l)

Case	Time: Hours −2	−1 1/2	−1	−1/2	Minutes 1	3	5	8	11	13	15
C1	0.44	0.54	0.68	0.63	0.55	0.48	0.46	0.46	0.56	0.76	0.91
C2	0.52	0.38	0.68	—	0.77	0.91	0.80	0.52	0.59	0.73	0.53
C3	0.42	0.43	0.40	0.48	0.59	0.50	0.42	0.37	0.39	0.46	0.50
C4	0.69	0.55	0.44	0.48	0.58	0.43	0.50	0.44	0.48	0.56	0.69
C5	0.32	0.30	0.24	0.32	0.29	0.29	0.26	0.24	0.43	0.48	0.49
C6	0.42	0.39	0.37	0.52	0.67	0.54	0.60	0.51	0.59	0.67	0.84
Mean	0.47	0.43	0.47	0.49	0.58	0.53	0.51	0.42	0.51	0.61	0.66
S.E.M	0.05	0.04	0.07	0.05	0.07	0.08	0.07	0.04	0.04	0.05	0.07

Table VI Plasma FFA concentrations during the experiment (mEq/l)

Case	Time: Hours −2	−1 1/2	−1	−1/2	Minutes 1	3	5	8	11	13	15
C7	0.77	0.69	0.64	0.82	1.16	1.03	0.90	0.97	0.96	1.08	1.17
C8	0.54	0.49	0.53	0.61	0.52	0.43	0.46	0.43	0.48	0.59	0.66
Mean	0.66	0.59	0.59	0.72	0.84	0.73	0.68	0.70	0.72	0.84	0.92

Table VII Plasma glycerol concentrations during the experiment (μmoles/l). The significance data for the controls C1—C6 (table IX) are given on the bottom line of the table

Case	Time: Hours −2	−1 1/2	−1	−1/2	Minutes 1	3	5	8	11	13	15
D1	49	59	67	58	77	92	106	127	139	—	132
D2	69	43	98	78	95	102	118	183	247	269	247
D3	46	29	67	87	118	118	131	201	322	327	310
D6	83	60	65	89	100	105	121	148	187	187	193
Mean	62	48	74	78	98	104	119	165	224	261	221
S.E.M	9	7	8	7	8	5	5	17	40	41	38
In comparison with C1—C6 p<	—	—	0.05	—	0.01	0.01	0.01	0.01	0.02	0.01	0.01

18	25	40	55	70	100
0 76	0 64	0 48	0 49	0 63	—
0 60	0 69	0 74	0 69	0 55	—
0 51	0 58	0 60	0 59	0 50	0 69
0 73	0 58	0 44	—	—	—
0 54	0 58	0 35	0 32	0 39	0 45
0 78	1 02	0 70	0 71	0 59	—
0 65	0 68	0 55	0 56	0 53	0 57
0 05	0 07	0 06	0 07	0 04	0 12

18	2ɔ	40	5ɔ	70	100
1 10	1 07	0 90	0 88	0 7ɔ	0 96
0 70	—	0 62	0 53	0 45	—
0 90	—	0 76	0 71	0 60	—

levels in comparison with the corresponding

18	2ɔ	40	55	70	100
116	98	73	60	42	51
233	217	113	93	70	74
273	233	107	62	6b	130
201	159	112	83	6ɔ	83
20b	177	101	75	61	8ɔ
33	31	10	8	6	17
0 01	0 01	0 02	—	—	—

higher values, but the difference is not significant

During exercise both the plasma FFA and the plasma glycerol concentrations in the diabetics of group A are higher than those in the controls The plasma glycerol level shows a pronounced rise which continues for some minutes after exercise After exercise the plasma FFA concentrations in the diabetics rise, and both the plasma glycerol and the plasma FFA levels are significantly higher than in the controls for up to 40 min after the beginning of exercise These findings are in good agreement with earlier findings in newly diagnosed juvenile diabetics (5 6 7), which were reported from this laboratory

The diabetics in group B do not differ from the controls of similar age in regard to the plasma FFA concentrations during and after exercise However, in regard to plasma glycerol, the mean concentration in the diabetic group is higher during exercise and for a considerable period after exercise The difference is significant at some points of time in spite of the low number of controls

It is generally agreed that the plasma glycerol concentration is a better indicator of the rate of lipid mobilization than the plasma FFA level (12, 16) Exercise initiates an increase of lipolysis in normal subjects (12) Judging from our earlier findings in newly diagnosed juvenile diabetics (5, 6, 7) and from group A individuals in the present study there is a more marked rise in lipid mobilization among the diabetics than in the controls In these diabetics there is a rather good correlation between the rises in plasma FFA and in plasma-glycerol

TABLE VIII Plasma glycerol concentrations during the experiment (μmoles/l) The significance data for the controls C7—C8 (table X) are given on the bottom line of the table

	Time										
	Hours				Minutes						
Case	—2	—1 1/2	—1	—1/2	1	3	5	8	11	13	15
D7	54	33	38	64	90	102	140	208	276	—	—
D9	—	49	63	59	89	103	123	190	214	225	218
D10	74	83	100	—	77	78	86	107	164	174	194
D11	59	62	91	104	115	110	121	146	276	324	344
Mean	62	57	73	76	93	98	118	163	233	241	252
S E M	6	11	14	14	8	7	11	23	27	44	47
In comparison with C7—C8											
p<	—	—	—	—	0 05	0 01	0 05	0 05	0 02	—	—

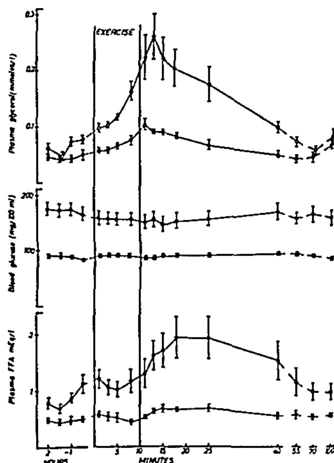


Fig 1 Plasma FFA blood glucose and plasma glycerol concentrations during the experiment in group A (○) and controls C1–C6 (●) Mean ± SE of mean

levels in comparison with the corresponding

18	25	40	55	70	100
306	229	87	52	61	79
216	178	80	44	37	70
187	152	82	73	71	—
331	272	185	120	59	76
260	208	109	72	57	75
35	27	26	17	7	3
0 02	0 02	—	—	—	—

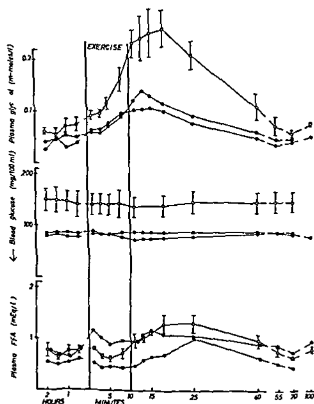


Fig 2 Plasma FFA blood glucose and plasma glycerol concentrations during the experiment in group B (○) and controls C7—C8 (●) In group B are shown mean ± SE of mean The individual curves are shown for the two control subjects

TABLE IX. Plasma-glycerol concentrations during the experiment (μmoles/l)

Case	Time Hours —2	—1 1/2	—1	—1/2	Minutes 1	3	5	8	11	13	15
C1	46	48	46	59	51	54	54	57	86	89	82
C2	43	49	67	—	67	63	66	76	95	92	89
C3	62	54	50	74	72	66	75	88	93	95	99
C4	60	45	31	34	47	47	65	70	145	79	89
C5	26	26	26	32	41	46	51	63	92	81	80
C6	40	35	35	54	70	75	81	101	115	109	107
Mean	46	43	43	51	58	59	66	76	104	91	91
S E M	6	4	6	8	5	5	5	7	9	4	4

TABLE X. Plasma glycerol concentrations during the experiment (μmoles/l)

Case	Time Hours —2	—1 1/2	—1	—1/2	Minutes 1	3	5	8	11	13	15
C7	27	49	30	41	64	66	78	97	104	104	107
C8	41	47	54	51	59	60	71	91	123	141	131
Mean	34	48	42	46	62	63	75	94	114	123	119

concentrations, account being taken of the fact that plasma FFA are consumed by the working muscles

In the diabetics of group B in the present study the correlation between plasma FFA and glycerol is poor as the plasma-glycerol level rises much more than the plasma FFA level during and after exercise There was no indication that this discrepancy was due to an impaired elimination of plasma glycerol in these older diabetics but more adequate studies of glycerol disappearance are needed before it can be excluded that in these diabetics in group B there is an impaired elimination rate of glycerol which explains the rise in plasma glycerol

Whilst the rise of plasma glycerol may well be due to a bigger increase in lipid mobilization during exercise this theory is disproved by the normal plasma FFA pattern unless it is assumed that a higher uptake of plasma FFA occurs in the exercised muscles — in which case the RQ values should be lower in group

18	25	40	55	70	100
80	51	45	42	70	—
84	75	57	41	42	—
96	92	74	66	63	91
71	51	34	—	—	—
74	54	38	29	31	48
107	91	68	47	36	—
85	69	53	45	48	70
6	8	7	6	8	22

18	25	40	55	70	100
102	79	49	36	41	50
118	93	61	47	47	—
110	86	55	42	44	50

B than in the groups of younger diabetics from the present and earlier (7) investigations No such difference was found In group A and in the younger diabetics studied earlier (7) a good correlation was found between the variations of plasma FFA and of plasma glycerol concentrations Thus further investigation is necessary to find the cause of this discrepancy

When the individual data for the diabetics were considered a trend was found that, in those who subsequently proved to need insulin therapy, plasma glycerol rose to higher levels than when oral therapy or diet alone proved an adequate The difference is, however not statistically significant

Summary

Newly diagnosed diabetics given no insulin, who developed the disease between the ages of 35 and 45, were compared with controls of the same age with regard to variations of plasma FFA and plasma glycerol concentrations during exercise Even at rest the plasma FFA and the plasma glycerol concentrations were higher in the diabetics and they rose after exercise indicating a more pronounced increase of lipid mobilization in response to exercise The variations of both the plasma FFA and the plasma glycerol levels followed the same pattern as in newly diagnosed, juvenile diabetics studied earlier

Newly diagnosed diabetics with the onset of disease after the age of 45 did not differ from controls of the same age in regard to plasma FFA levels during the experiment The plasma glycerol concentrations in the diabetics rose to significantly higher levels than in the controls during and after exercise The possible causes of this discrepancy between the two indicators of lipid mobilization are discussed

Acknowledgements

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TABLE XI Blood glucose concentrations during the experiment (mg/100 ml) Mean and S E. Mean and range for the controls C7—C8

		Time										
		Hours				Minutes						
		—2	-1 1/2	—1	—1/2	1	3	5	8	11	13	15
Group A (cases	Mean	174	172	173	165	157	156	155	155	150	155	146
D1—D6)	S E M	12	12	11	12	13	10	11	11	12	14	13
Group B (cases	Mean	151	149	146	141	140	143	141	143	137	120	120
D7—D11	S E M	21	25	19	25	20	20	19	27	20	13	14
Cases	Mean	90	90	89	82	90	91	91	90	86	87	90
C1—C6	S E M	4	5	3	2	2	3	3	4	4	3	3
Cases	Mean	82	84	83	82	87	84	84	83	82	82	82
C7—C8	Range	81-83	83-84	79-86	79-84	84-89	84-84	84-84	78-88	74-89	75-88	75-88

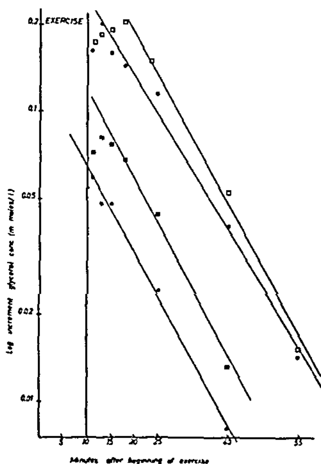


Fig 3 Log mean increment glycerol concentration in the period after exercise for group A (○), control subjects C1—C6 (●) group B (□) and control subjects C7—C8 (■) plotted against time in minutes.

for group A group B and the controls C1—C6

18	25	40	55	70	100
150	156	169	156	165	159
12	13	15	11	14	15
140	147	145	146	145	117
21	22	20	21	19	8
91	92	95	92	91	85
3	3	3	3	3	3
83	84	86	87	84	78
78–88	79–89	84–88	86–87	83–85	—

TABLE XII RQ values in the subjects examined at rest and during exercise No significant differences are found between group A and controls C1—C6 or between group B and controls C7—C8 either at rest or during exercise

Group	Case	At rest	During exercise
A	D1	0.82	0.90
	D2	0.71	0.90
	D3	0.71	0.89
	D4	0.79	1.06
	D5	0.72	0.88
	D6	0.97	0.92
	Mean ± S E M	0.79 ± 0.041	0.93 ± 0.026
B	D7	0.73	0.80
	D8	—	—
	D9	0.74	0.80
	D10	0.83	0.78
	D11	—	0.89
	Mean ± S E M	0.77 ± 0.032	0.82 ± 0.025
C1—C6	C1	0.74	0.85
	C2	1.01	0.90
	C3	0.77	0.95
	C4	0.83	0.89
	C5	0.96	0.90
	C6	0.76	0.80
	Mean ± S E M	0.85 ± 0.046	0.88 ± 0.021
C7—C8	C7	0.81	0.90
	C8	0.73	0.92
	Mean	0.77	0.91

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Mediastinal Lymphosarcoma with Superior Vena Cava Syndrome and Restrictive Heart Disease

Report of a case

By

A BJERMULF, L BJORK, I CULLHED and E ENGHOFF

Lymphosarcomas only rarely become manifest as a vena cava superior syndrome a serious complication of this malignant disease Where obstruction of the superior vena cava is in fact the initial symptom heart involvement is commonly found at autopsy but the clinical diagnosis of cardiac involvement i not often made

The purpose of this paper is to present a case of lymphosarcoma in which the initial manifestation of disease was signs and symptoms typical of superior vena cava obstruction also with clear cut clinical evidence of cardiac involvement The case was repeatedly subjected to angiocardiography

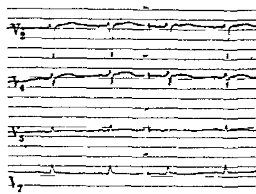
Case report

The patient was a 35 year old man previously healthy In 1964 he probably had a superficial thrombophlebitis of the chest wall In June 1965 he got a cold followed by cough and right sided pleuritic pain He gradually became breathless and had to sleep in a sitting position At admission he was in acute distress with pronounced breathlessness and cyanosis of the upper half of the body The neck veins were distended The left arm was swollen and oedematous with a circumference over the biceps muscle of 32 cm compared with 28 cm on the right The temperature was 38 5° C The B P was 105/70 mm Hg the pulse paradoxic and regular with a frequency of 120/min The heart sounds were faint no third sound was audible A faint systolic murmur was heard but no pericardial friction rub The examination of the lungs revealed bilateral rhonchi and rales right sided friction rubs and signs of bilateral effusion The liver was slightly enlarged

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Laboratory data

At admission the patient had normal values of Hb and Hct but a moderate leucocytosi (88 5 % polymorphnuclears some with toxic granulations 6 5 % lymphocytes and 5 % monocytes) There were no eosinophils basophils or any immature cells The ESR was 25 mm/hr and non protein nitrogen 42 mg% Cultures from blood bone marrow and pleural fluid were sterile Cytologic examination of the pleural effusion failed to show tumor cells Sternal marrow was normal



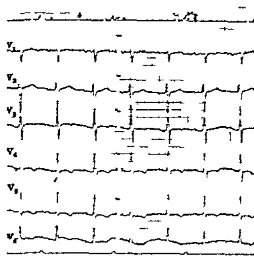




Fig 1 ECGs

A At admission flat T waves in left precordial leads and slight ST depression probably secondary to digitalis therapy
B A month later negative T waves in left precordial leads
C About three and a half months after admission ST elevation and low voltage

Mediastinoscopy revealed oedema and a firm tumour mass on the left side of the trachea just above the main bronchus Histopathological examination revealed occasional atypical cells These could be consistent with a necrotized malignant tumour but did not permit a firm diagnosis There was no bleeding during or after this procedure

ECG

There was constant sinus tachycardia except for transient atrial fibrillation during a few days. At admission (fig 1 A) ECG showed flat T waves in left precordial leads and slight ST depression probably secondary to digitalis therapy A month later (fig 1 B) there appeared negative T waves and later (fig 1 C) low voltage and ST elevation

Roentgen studies

Chest X ray at admission showed a tumour mass in the anterior superior mediastinum The heart size could not be calculated because of large bilateral pleural effusions

Cavography a few days after admission (fig 2 A) showed a total occlusion of the left innominate vein and both jugular veins There was a pronounced stenosis of the superior vena cava with a diameter of only 3 mm The calibre on the head side of the stenosis was 20 mm in both projections There was also a compression of the pulmonary artery main stem





A B

Fig 2 A Cavography before radiotherapy Severe stenosis of superior vena cava (←———) and compression of the pulmonary artery main stem (←– – –)
B Cavography after radiotherapy only moderate stenosis of superior vena cava (SVC) and a normal pulmonary artery (PA)

The mean pressure in the vena cava on the head side of the stenosis was 26 mm Hg and in the right atrium 8 mm Hg A month later when he had received radiotherapy a second cavography was performed (fig 2 B) The diameter of the stenotic part of the superior vena cava had increased significantly compared with the first cavography and there was no compression of the pulmonary artery The pressure gradient over the stenosis was only 5 mm Hg The initial widening of the mediastinum was no longer found

Further course and treatment

The patient's general condition rapidly improved after frequent pleurocenteses Pericardial puncture yielded only 3 ml of yellow transparent fluid A pericardial friction rub was first noticed a week after admission and then persisted A third heart sound became audible Treatment was continued with prednisone in an initial dose of 60 mg daily as well as with furosemide and digitalis After a fortnight the patient received the first series of radiotherapy which resulted in marked subjective and objective improvement for about four weeks In the further course however there was a consistent deterioration in spite of two further series of radiotherapy We tried cyclophosphamide without benefit Large pleural effusions necessitated repeated punctures During the last months he had distressing pains in his right chest and abdomen which became distended with hepatomegaly and ascites Roentgenologically there was a slight splenomegaly The superficial lymph nodes remained normal There were weekly attacks of fever lasting two or three days He died in Jan 1966 about six months after the disease began



Fig 3 The heart at autopsy The myocardium is infiltrated by tumour tissue with yellow white cut surface At the border between tumour and normal myocardium there is a haemorrhagic zone



Fig 4 Microscopy The myocardium is infiltrated by lymphoid cells mostly lymphocytes but also lymphoblasts

Autopsy findings (autopsy performed by Dr I Veylens Dept of Pathology University of Uppsala)

Macroscopy (fig 3

The heart was enlarged weighing 650 g The pericardium was totally adherent to the heart it was firm and thick infiltrated by metastases with a hard consistency The myocardium was infiltrated by tumour masses whose cut surface was yellow white At the border between tumour and normal myocardium there was a haemorrhagic zone The ventricular and atrial septa were not involved The coronary arteries could not be dissected more than a few cm due to the surrounding tumour tissue

The first part of the ascending aorta was embedded in tumour growth but otherwise seemed to be normal There was possibly some narrowing of the pulmonary veins where they entered the left atrium Both the superior and the inferior venae cavae were narrowed by the tumour The pulmonary arteries appeared normal The mediastinal lymph nodes were invaded by tumour growth but the hilus nodes were normal The right but not the left lung was adhesive to the chest wall with pleural tumour over growth The diaphragm too was invaded by tumour masses

The liver weighing 2 000 g showed stasis The spleen with rather firm parenchyme had a weight of 200 g In the stomach and duodenum there were four small ulcers The tumour infiltrations descended around the abdominal aorta

Microscopy (fig 4)

Sections from the mediastinal tumour masses showed the tumour to be composed of lymphoid cells Many resembled lymphocytes but there were also many lymphoblasts The same microscopic picture was found in sections from the pleura pericardium myocardium abdominal lymph nodes and spleen No tumour cells were found in the liver

Discussion

The symptoms and signs of superior vena cava obstruction are related to increased venous pressure on the head side of the lesion, and are aggravated, as in our patient, by lying down The severe breathlessness could be due to the peri cardial and myocardial involvement, as well as the pleural effusions, which might be secondary to narrowing of the pul monary veins The only slight increase in right atrial mean pressure speaks against cardiac constriction as the cause of the hydrothorax The patient had oedema of his left arm The left side is more frequently affected perhaps due to the longer mediastinal course of the left innominate vein There was no distension of the superficial veins in the thorax and abdomen This possibly indicated that the obstruction was above the azygos vein Other symptoms were dysphagia and hoarseness due to oedema in the laryngeal regions Often these patients have a severe headache due to raised intracranial pressure, as demonstrated by Ask Upmark (1)

As mentioned previously we performed cavographies and measurements of the pressure gradient over the stenosis to localize the obstruction and to study the result of radiotherapy Venous angiography is the best method to visualize the obstruction (6 8)

Concerning the aetiology of the superior vena cava syndrome, McIntyre and Sykes (5) found primary intrathoracic malignant tumours to be responsible for the obstruction in 29 6 % in a collection of 250 cases Kalter et al (3) found it in only seven of 100 cases of mediastinal tumours of which three turned out to be malignant In other series of cases of mediastinal tumours malignancy has been found in 32 % (2, 4) They were lymphosarcomas in 3 3 % (4) and 7 4 % (2) respectively

In our patient there were no clinical or laboratory data suggesting tuberculosis, syphilis or histoplasmosis nor any previous pericarditis No X ray treat ments had been given earlier The rapid progressive course of the disease sug gested a malignant process A bronchial carcinoma could not be excluded despite the localization of the tumour to the anterior mediastinum Mediastinoscopy showed a tumour but no definite his tologic diagnosis was possible The strong suspicion of a malignant disease in our patient was increased by the good re sponse to the first roentgen treatment as visualized on the cavographies

In our case there were early signs of cardiac involvement (pericardial fric tion rub, third heart sound and ECG changes typical of peri myocarditis) At autopsy there was concentric growth of tumour tissue on both pericardium and myocardium Stationary electrocardiographic changes in lymphosarcoma are difficult to evaluate as they may be due to unrelated heart disease

Rosenberg et al (7) in a review of 1 269 patients with verified lymphosar coma found clinical signs of intrathoracic involvement in 50 8 % Only 4 % pre sented with initial symptoms of intra thoracic lesions In their series 2 1 % showed the superior vena cava syndrome which was seen as the initial manifesta tion in 16 cases According to these authors the incidence of heart involve ment varies from 17 to 27 % in different

series Among their 277 autopsied patients, 63 cases had gross or microscopic deposits of tumour in the heart However, in only nine patients was cardiac engagement suspected before death The pericardium was affected in eight cases, epicardium and pericardium in 35, myocardium in 39, and endocardium in seven cases

Concerning the treatment, Rosenberg et al (7) considered radiotherapy to be the therapy of choice for lymphosarcomas They found no increase in survival over a period of 25 years despite the addition of steroids and chemotherapy Urschel and Paulsen (8), however, in a recent report of 61 patients with superior vena cava obstruction, had significantly better results with nitrogen mustard followed by radiotherapy, than with radiotherapy or nitrogen mustard alone

Summary

A case of mediastinal lymphosarcoma is reported presenting large pleural effusions and signs of cardiac involvement along with the superior vena cava syndrome Mediastinoscopy could be performed without bleeding By cavographies and central venous pressure measurements, we could localize the obstruction and objectively demonstrate the good initial result of radiotherapy Autopsy findings verified the clinical signs of intracardiac involvement

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From Medical Department IV Sodersjukhuset and Maria Outpatient Service for Alcoholism Stockholm Sweden

Serum Creatine Phosphokinase in Chronic Alcoholism

By

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In chronic alcoholism two different forms of muscular disorder have been described, namely, an acute muscular syndrome with pain tenderness and oedema in the musculature, and a chronic muscular syndrome characterized by slowly progressing proximal muscular weakness (3, 4 5 6 12 22 28) Increased serum values of the specific muscular enzyme creatine phosphokinase have also been observed in acutely intoxicated alcoholics (16 17, 19, 29)

In this investigation serum creatine phosphokinase (CPK) glutamic oxalo acetic transaminase (GOT) and glutamic pyruvic transaminase (GTP) have been measured in nine alcoholics for 8—14 days after an alcoholic debauch Two groups with different enzyme patterns were noted one group with a picture similar to that seen in muscle damage, and the other with a combined muscle and liver enzyme pattern

Material

The material consisted of nine men aged 32—54 years who were admitted to a ward for alcoholics on account of acute alcoholic intoxication. All had been addicts for a number of years and had been treated in institutions or mental hospitals for alcoholism The nine patients were selected at random from alcoholics who on admission had had raised serum CPK activities

Shortly before admission all the patients had indulged in an alcoholic debauch during a period varying from one week to two months Their daily consumption of strong spirits had been between 1/4 and 1 1/2 l (in case 5 whisky the remainder corn brandy) Two patients (cases 1 and 2) had drunk in addition 1—2 bottles of wine daily

Four patients (cases 3 7 8 and 9) had not eaten anything for 4—7 days immediately before admission. Five patients (cases 1 2 4 5 and 6) had eaten at most sporadically about two cooked meals per week

One patient (case 7) had felt pain and tenderness in the muscles of his calf whereas the others had no subjective muscular complaints

None of the patients displayed any noteworthy physical signs The liver was not palpable and deep tendon reflexes sensibility and muscular strength in the extremities were normal in all the patients All had normal ECGs (standard leads and chest leads CR_1)

Bilirubin alkaline phosphatase and thymol turbidity were normal in all cases

Paper electrophoresis of serum proteins

Submitted for publication March 16 1967

was carried out in four cases (nos 1, 3, 6 and 7) In case 6 the a_1 and a_2 globulins were slightly raised, and in case 7 the a_2 fraction was somewhat increased In cases 1 and 3 the electrophoresis pattern was normal

Liver biopsies were performed in five cases (nos 2, 4 5 7 and 8) In cases 4, 5 and 7 there was fatty degeneration of the liver In cases 2 and 8 the liver biopsy findings were normal

Methods

GOT and GPT were determined according to Reitman and Frankel (21)
Normal values
GOT 0—40 units
GPT 0—35 units
The serum was kept in a refrigerator until determinations were made within 48 hours

CPK was determined according to Hughes (10) The serum was kept frozen (—20° C) and determinations were made within two weeks Normal values in this laboratory for hospitalized patients were 0 3—4 1 units (17) Other laboratory tests were made by standard methods

Liver biopsy specimens were obtained with a Vim Silverman needle

Results

Serum enzyme values

Four patients (cases 1—4) had raised serum CPK and GOT activities where as serum GPT was normal (fig 1 and table I) In these cases, elevated CPK values were found during a period of from 5 to 7 days Two patients (cases 1 and 3) did not display any normal CPK values during their hospital stay In all patients in this group serum GOT values became normal at least two days before the CPK values

In five patients (cases 5—9) serum CPK, GOT and GPT values were raised

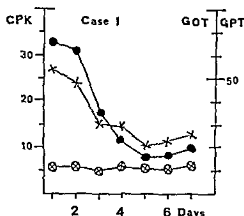


Fig 1 Case 1 Serum CPK (●—●) GOT (×—×) and GPT (⊗—⊗)

(table I) In this group elevated CPK values were found for periods ranging from 3 to 10 days In two patients (cases 6 and 8) the raised serum GOT persisted longer than the raised CPK In the other cases the sequence in which the serum CPK and GOT returned to normal was not determined Serum GPT was increased for a longer time than serum GOT, except for case 8, where no normal values for these enzymes were recorded

Muscle biopsy

Muscle biopsy from the musculus quadriceps was performed in cases 1, 2, 3 4, 5 and 6 In case 3 there was slight general atrophy In case 6 one or two segmentally destroyed fibres were observed with single inflammatory cells In the other cases no histologic changes were seen

Discussion

In this series the highest serum CPK activities were between 12—65 units The highest recorded normal value for

TABLE I Serum CPK, GOT and GPT in nine acutely intoxicated alcoholics with raised CPK values. Normal values CPK 0.3—4.1 GOT 0—40 GPT 0—35 units

Cases		Days after admission										
		1	2	3	4	5	6	7	8	8	10	14
1	CPK	33	31	17	12	8	8	10	—	—	—	—
	GOT	54	48	30	28	21	22	26	—	—	—	—
	GPT	12	12	11	12	12	11	13				
2	CPK	33	20	13	19	11	—	4.5	3.0	—	—	—
	GOT	61	60	32	40	22	—	18	13	—	—	—
	GPT	33	30	24	21	18	—	13	12	—	—	—
3	CPK	32	31	13	11	9	7.5	—	—	—	—	—
	GOT	57	56	28	22	20	20	—	—	—	—	—
	GPT	24	26	15	12	10	10	—	—	—	—	—
4	CPK	12	6	9	—	4.5	2.8	—	—	—	—	—
	GOT	42	26	31	—	18	17	—	—	—	—	—
	GPT	21	21	20	—	15	13	—	—	—	—	—
5	CPK	48	20	11	5.5	—	—	—	2.3	3.8	—	—
	GOT	101	94	76	70	—	—	—	33	38	—	—
	GPT	42	52	52	49	—	—	—	36	42	—	—
6	CPK	25	17	9	—	9	8.5	—	2.5	1.5	3	—
	GOT	93	75	64	—	44	43	—	40	38	39	—
	GPT	59	52	49	—	37	35	—	41	42	45	—
7	CPK	65	—	27	—	—	8.7	—	6.0	—	8.1	3.3
	GOT	350	—	260	—	—	82	—	57	—	48	21
	GPT	450	—	420	—	—	203	—	131	—	104	48
8	CPK	—	—	17	—	37	—	—	—	—	—	3.5
	GOT	108	—	84	—	93	—	—	—	—	—	44
	GPT	128	—	120	—	109	—	—	—	—	—	48
9	CPK	20	21	18	—	—	—	—	3.3	—	—	—
	GOT	60	64	54	—	—	—	—	32	—	—	—
	GPT	30	34	40	—	—	—	—	36	—	—	—

28 hospitalized patients was 4.1 units (17). Other investigators who applied a similar method found normal values of the same order of magnitude (2, 10, 14, 18). Thus, a significant pathological increase in serum CPK was observed in all nine patients.

In four patients (cases 1—4) serum CPK and GOT were raised. Serum CPK activity in these four patients was raised for 5 to 7 days and the GOT returned to normal some days before the CPK value. In cases 2 and 3 a gradual decrease of the GPT value was noticed during the hospital stay. However, all GPT values were within the normal

range in these two cases and in cases 1 and 4. In liver disease elevated GOT values in combination with normal GPT activity usually occurs only in cirrhosis or in primary or metastatic carcinoma of the liver (11, 24, 30). No patient in this series had any signs indicating liver cirrhosis or hepatic malignancy. It is therefore probable that in these cases the increased enzyme release into the extracellular compartment was due mainly to damaged muscle cells.

In five patients (cases 5—9) the serum CPK, GOT and GPT were elevated. When such an enzyme pattern occurs in myocardial infarction it is thought to be due to hepatic anoxia in addition to the myocardial injury (20, 30). However, elevations of GPT have been reported in the acute phase of muscular dystrophy and this has been interpreted by some investigators as an expression of the muscular damage (26, 27). In fact hepatic involvement has been reported in muscular dystrophy and the elevations of GPT in muscular dystrophy may therefore be due to liver damage (9, 13). Thus it is possible that the elevated GPT values in cases 5—9 were due to increased release of the enzyme from liver cells, especially as liver disease is one of the hallmarks of chronic alcoholism.

The enzyme patterns in this series have some similarities with that seen in myocardial infarction. However, the CPK remained elevated longer than in myocardial infarction, where it usually returns to normal within 3—4 days (8, 15, 23, 25). Moreover in the group with a 'pure' muscular pattern the GOT returned to normal before the CPK, whereas in myocardial infarction the CPK usually returns to normal first (8, 15, 23, 25). Accordingly it is evident that in these patients the serum CPK pattern differed from that observed in myocardial infarction.

Histological examination of the biopsy material from the musculus quadriceps did not show unequivocal acute muscle cell necrosis in any of the cases investigated. The significances of the changes in case 6 are questionable since necrosis of single fibres can be found in specimens from patients without known muscle disease (1). However, muscle cell necrosis may have been present in other parts of the skeletal musculature. A cell can lose intracellular enzymes into the extracellular space without dying and this loss seems to be a direct consequence of a lesion affecting the energy yielding reactions of the cell (7).

In this connection Perkoff et al. (19) observed a subnormal blood lactic-acid response in ischemic exercise in alcoholics when in a condition of acute intoxication. They found that this disturbance continued for about a week after the debauch had terminated, which is about the same length of time as raised CPK values persisted in this series. Consequently, it is conceivable that the raised serum CPK following an alcoholic debauch may be caused by a general disturbance in the energy metabolism of the muscle cells, which in turn leads to increased cell permeability and thereby causes an inhanced release of the enzyme CPK into the extracellular space.

Summary

The serum activities of CPK, GOT and GPT were followed in nine alcoholics

with acute intoxication and raised CPK values Two enzyme patterns were distinguished a muscle enzyme pattern, and a picture indicating enzyme release from muscle as well as liver cells

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The 10th International Congress of Internal Medicine will be held in Warsaw from September 10 to 14, 1968

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The Influence of Depth of the Cut on the Bleeding Time

By

PER STAVEM

The bleeding time is important for the diagnosis of von Willebrand s disease and platelet disorders Duke s bleeding time in the lobe of the ear (5) was in general use until Ivy et al introduced the venous pressure bleeding time (6) a method which has since been preferred by many workers Recently it was shown however that infusions of plasma or Cohn s fraction I markedly affect the Duke but not the Ivy bleeding time in von Willebrand s diesase (2) Since such infusions protect these patients against surgical bleeding, the Duke bleeding time may be the better clinical guide (2)

In both methods the technique markedly influences the bleeding time A sharp knife gives longer bleeding than a blunt one A wide cut bleeds longer than a deep narrow cut because the elasticity of the skin tends to mechanically seal a narrow wound Most workers have used new surgical blades of the type Bard Parker no 11 (English Swann Morton no 11) The depth and width, however have varied greatly from a deep and narrow cut made with the blade perpendicular to the skin surface (3 8) to a shallow and wider cut made with the cutting edge almost parallel to the skin surface (1, 2) This is illustrated in fig 1 Spring lancets (6 7), capillary prickers or Hagedorn needles (4) produce narrow cuts of varying depth

We suspected that the bleeding time varies with the depth of the cut when the cutting edge is held parallel to the skin surface We have now studied influence of the depth of the cut on both the Duke and the Ivy bleeding times in normal persons and in patients with various platelet disorders Our data show that deeper wounds bleed much longer the bleeding time technique should therefore be carefully standardized

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Methods

Duke bleeding time

The ear lobe was first wiped with alcohol dried with gauze and gently rubbed between two fingers for 15 sec The lobe was then lightly compressed between the two blades of a hemostat forceps leaving a free brim of 2 mm protruding beneath the

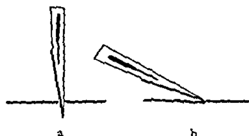


Fig 1 Cut with the cutting edge respectively perpendicular to (a) and parallel to skin surface (b)

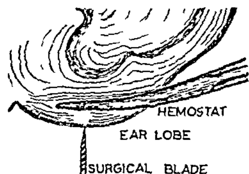


Fig 2 Measuring out a free brim of 2 mm beneath the hemostat blades and then cutting right down to the hemostat with the surgical blade

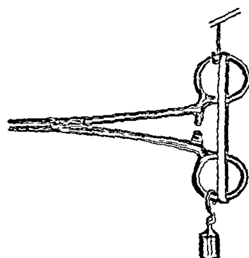


Fig 3 Selecting a rubber band which compresses the hemostat blades with a pressure equal to the gravitational pull of 100 g

blades for cuts 2 mm deep (fig 2) The protruding brim was then cut right down to the hemostat with a new Swann Morton no 11 surgical blade A free brim of 1 mm was allowed to protrude for cuts 1 mm deep The protruding brim of ear lobe was adjusted by comparison with an engraved line on the hemostat blade (fig 2) The line was engraved at the point where the hemostat blades had a cross measure of exactly 1 mm which was about 3 mm from the tip (fig 2) The blades of the hemostat were lightly pressed against the ear lobe by a rubber band selected to give a pressure of about 100 g (fig 3) When the incision had been made, the hemostat was removed and the drop of blood hanging below the cut was blotted with filter paper each half minute Care was taken not to disturb the wound The area of the blot after the first half minute has been expressed by its diameter The pressure of the hemostat blades applied for a few seconds before and during the incision did not influence the bleeding time

Ivy bleeding time

A similar procedure was used for the Ivy method A small skin fold on the volar side of the forearm was picked up and allowed to protrude 1/2 and 1 mm above the hemostat blades for cuts 1/2 and 1 mm deep respectively A blood pressure cuff around the upper arm was kept inflated to 40 mm Hg during the test The wound was blotted every 1/2 minute, as for the Duke bleeding time

Material

Duke and Ivy bleeding time tests with wounds of various depths were carried out in ten healthy nurses ten healthy doctors and 20 patients with thrombocytopenia More limited studies were performed in an additional 28 healthy persons and six thrombocytopenic patients Only one cut was made for each depth

TABLE I Bleeding times from cuts of different depths in randomly selected healthy nurses and doctors

	Age	Duke Blot diam (cm)	1 mm Bleed time (min)	Duke Blot diam (cm)	2 mm Bleed time (min)	Ivy 1/2 mm Bleed time (min)	Ivy 1 mm Bleed time (min)	Thrombocytes × 1 000	Hb (g/100 ml)
Women									
SJ	22	0 1	1/2	1 0	9	4 1/2	6	197	14 1
HH	26	0 1	2	1 8	12	5	9	221	12 8
AK	26	0 4	4	1 7	14	10 1/2	17	218	12 4
LN	28	0 1	2	0 9	5	4 1/2	5 1/2	297	14 6
IF	29	0 4	3 1/2	1 1	12	9 1/2	7 1/2	248	13 3
RH	31	0 1	1/2	0 6	7	5 1/2	4	253	13 7
RF	39	1 0	4	2 3	8 1/2	4	12	212	12 1
AL—R	42	0 3	3	1 0	15	4	16	145	13 3
SM	45	0 4	3	1 1	25 1/2	12 1/2	27 1/2	196	12 7
TB	53	0 2	2	1 0	9 1/2	1	6	226	15 0
Median	30	0 25	2 1/2	1 05	10 3/4	4 3/4	8 1/4	220	13 3
Men									
KG	32	0 1	2	1 0	8	2 1/2	11 1/2	194	16 6
MJ	32	0	0	0 7	6	1 1/2	5	189	16
AN	33	0 3	2	0 65	5	5	8	215	16 7
TR	33	0	0	0 8	9 1/2	5	5	201	15
NS	33	0 4	3	1 9	8 1/2	5 1/2	6 1/2	215	15 6
EM	35	0 1	1	1 7	9	5	7	197	16 7
DJ	36	0 2	2 1/2	2 3	13	4	3	233	14 1
BK	36	0 3	2	0 8	6	5	4 1/2	235	15 6
PH	42	0 5	2	1 6	4 1/2	6 1/2	6 1/2	205	14 6
AB	50	0 1	1	1 0	6	2	4	192	15 3
Median	33	0 15	2	1 0	7	5	5 3/4	203	15 6

Results

Tables I and II detail the results obtained in 20 normal persons and in 20 thrombocytopenic patients

Table III shows that the median of ten normal persons did not differ unduly when the Ivy bleeding times from a cut 1/2 mm deep were compared with those from a cut 1 mm deep However some normal persons bled much longer when the cut was made deeper For ten normal men the longest bleeding time from a cut 1/2 mm deep was 6 1 2 minutes whereas three of them bled for 7 8 and 11 1/2 minutes respectively from a cut 1 mm deep (table I) For ten normal women the longest bleeding time from a cut 1 2 mm deep was 12 1 2 minutes whereas three of them bled for 16, 17 and 27 1/2 minutes, respectively from a cut 1 mm deep

Table IV shows that the median Duke

TABLE II Bleeding times from cuts of different depths in patients with thrombocytopenia

	Age	Diagnosis	Duke 1 mm		Duke 2 mm	
			Blot diam (cm)	Bleed time (min)	Blot diam (cm)	Bleed time (min)
Women						
AB	57	Leukemia	1 8	>30	—	—
IL	58	ITP	0 1	1	1 0	>30
GS	32	Leukemia	0 4	6	1 7	27 1/2
AH	20	Hered T P	0 4	23	0 9	>30
RK	37	Mb Gauchet	0 6	7 1/2	1 2	14 1/2
RS	59	Pancytopen	0 4	3 1/2	1 4	>30
ES	51	Pancytopen	0 1	1/2	1 3	16 1/2
PO	72	Lymphoma?	0 4	4	0 9	11 1/2
LA	63	Lymphoma?	0	0	1 1	13
Median	57		0 4	4	1 2	27 1/2
Men						
FB	59	ITP	0 2	2	1 0	>30
SM	16	Leukemia	0 6	15 1/2	3 3	>30
RE	43	Leukemia	0 4	8 1/2	1 4	>30
OS	69	Leukemia	0 1	1	0 5	9 1/2
AJ	64	Leukemia	0 5	8 1/2	1 5	28 1/2
JK	58	Leukemia	0 2	2 1/2	1 2	>30
OL	64	Leukemia	0 4	2	2 3	9 1/2
GM	55	ITP	0 4	2	2 3	14 1/2
KO	57	Leukemia	0 1	1 1/2	1 2	20 1/2
KS	66	Leukemia	0 3	1	2 1	16 1/2
KH	17	Leukemia	0 8	10	3 1	>30
Median	58		0 4	2	1 5	28 1/2

TABLE III Ivy bleeding time from cuts of different depth in normal persons and in patients with thrombocytopenia (Figures from tables I and II)

	Ivy 1/2 mm deep		Ivy 1 mm deep	
	Range	Median	Range	Median
Ten normal women	1—12 1/2	4 3/4	4—27 1/2	8 1/4
Ten normal men	1 1/2—6 1/2	5	3—11 1/2	5 3/4
Ten thrombocytopenic women			20 1/2—>30	>30
Ten thrombocytopenic men			16 1/2—>30	>30

Ivy 1/2 mm Bleed time (min)	Ivy 1 mm Bleed time (min)	Thrombocytes × 1 000	Hb (g/100 ml)
	>30	3 7	9 8
	>30	11 7	14 1
	>30	20	7 1
	>30	26	15
	20 1/2	30	8 6
	>30	49	10 3
	>30	56	9 5
	23 1/2	80	12 1
	27 1/2	113	10 3
	>30	30	10 3
	>30	1 2	12 4
	>30	5	8 2
	>30	11	14 3
	>30	12	8 7
	>30	15	8 7
	>30	18	9 5
	21	41	10 1
	16 1/2	65	16
	>30	76	9 9
	19 1/2	76	11 1
	>30	82	8 4
	>30	8	9 9

TABLE V Bleeding times from 2 mm deep cuts in the ear lobe and 1 mm deep cuts on the forearm in 8 randomly selected healthy student nurses

	Age	Duke 2 mm Bleed time (min)	Ivy 1 mm Bleed time (min)
GL	18	5 1/2	10 1/2
IG	19	8	10 1/2
LS	20	7 1/2	6 1/2
KB	20	13 1/2	18 1/2
IF	20	7	16
KJ	20	25 1/2	12
AE	21	5	8
RG	21	5	10
Median	20	7 1/4	10 1/2

bleeding time from cuts 2 mm deep was about four times longer than the median from cuts 1 mm deep The deeper cuts also resulted in some really long bleeding times in some normal women as did the deeper cuts with the Ivy method From a 2 mm deep cut in the ear lobe three out of ten normal women bled for 14 15 and 25 1/2 minutes respectively The women with the longest Duke bleeding

TABLE IV Duke bleeding time from cuts of different depth in normal persons and in patients with thrombocytopenia (Figures from tables I and II)

	Duke 1 mm deep		Duke 2 mm deep	
	Range	Median	Range	Median
Ten normal women	1/2—4	2 1/2	5 1/2—25 1/2	10 3/4
Ten normal men	0—3	2	4 1/2—13	7
Ten thrombocytopenic women	0—>30	4	11 1/2—>30	27 1/2
Ten thrombocytopenic men	1—15 1/2	2	9 1/2—>30	28 1/2

TABLE VI Ivy bleeding times from cuts of different depths in patients with thrombocytopenia

	Sex Age	Diagnosis	Ivy 1/2 mm Bleeding time (min)	Ivy 1 mm Bleeding time (min)	Thr cytes × 1 000	Hb (g/100 m)
NB	♀ 16	ITP	>30	>30	5 6	11 4
SB	♀ 67	ITP	>30	—	11 7	14 1
IL	♀ 58	ITP	25	>30	35	14 1
MB	♂ 27	ITP	>30	—	7 3	17 3
DG	♂ 48	ITP	24 1/2	>30	12 4	16 3
AR	♂ 11	Hered TP	>30	—	22	12 7

TABLE VII The range median mean and standard deviation (S D) of individual Ivy bleeding times from one 1/2 mm deep cut in 20 normal women and from one 1 mm deep cut in 20 normal men All values in minutes

	Ivy 1/2 mm deep				Ivy 1 mm deep			
	Range	Median	Mean	S D	Range	Median	Mean	S D
Twenty normal women	1 13 1/2	7	7 5	3 1/2				
Twenty normal men					3—13	5 1/2	6 4	2 1/2

times from cuts 2 mm deep also had the longest Ivy bleeding times from cuts 1 mm deep

Because of the markedly prolonged bleeding from the deep cuts in three of the healthy women in table I, a study limited to deep cuts were done in another eight healthy women Table V shows that the women with the longest Duke bleeding times from cuts 2 mm deep also had the longest Ivy bleeding times from cuts 1 mm deep

In the original 20 thrombocytopenic patients the Ivy bleeding time was tested only with cuts 1 mm deep Another six thrombocytopenic patients were therefore studied with cuts 1/2 mm as well as 1 mm deep Table VI shows that all six thrombocytopenic patients bled for 24 1/2 minutes or more from cuts 1/2 mm deep Table VII gives the range, mean and standard deviation of individual Ivy bleeding times from one 1/2 mm deep cut in 20 normal women and from one 1 mm deep cut in 20 normal men

Discussion

The results show that the depth of the cut is important for the bleeding time, at least when the cut is made with the cutting edge almost parallel to the surface of the skin

The median Ivy bleeding time of normal persons increased only slightly when the depth of the cut was increased from

1/2 mm to 1 mm However, some normal persons have a prolonged bleeding time when the cut is 1 mm deep From our results, it seems that cuts 1/2 mm deep more accurately separate normal women from women with a bleeding tendency than do 1 mm cuts In men, one might choose either 1/2 or 1 mm deep cuts, but should be aware that cuts 1 mm deep occasionally bleed for more than 10 minutes in normal men

The median Duke bleeding time from cuts 2 mm deep was about four times longer than the median from cuts 1 mm deep The deeper cuts also resulted in some really long bleeding times in a number of normal women, as did the deeper cuts with the Ivy method The median Duke bleeding time from cuts 1 mm deep in the thrombocytopenic patients was only slightly longer than that *for normal persons*, showing that a cut as shallow as 1 mm will not differentiate normal persons from persons with a bleeding tendency Cuts 2 mm deep on the other hand seem to be somewhat too deep in women as five out of 18 normal women bled for 14 or more minutes Even in men the Duke bleeding time from cuts 2 mm deep is less suitable for differentiating between normal persons and persons with a bleeding tendency than the Ivy bleeding time from a cut 1/2 mm deep On the other hand when the Duke bleeding time from a 2 mm deep cut in the ear lobe exceeds 30 minutes it might carry a greater significance than when the Ivy bleeding time from a 1 2 mm deep cut exceeds 30 minutes

The first half minute s outflow of blood from a 1 mm deep cut in the ear lobe was in almost all cases quite small much smaller than recommended by Duke (5) Duke recommended that the cut should be of such a size that the first half minute s outflow of blood made a blot 1—2 cm in diameter Duke also stated that within certain limits the bleeding time did not depend on the size of the cut, but he only tried cuts large enough to give a blot of more than 1 cm in diameter within the first half minute In most of our cases, 2 mm deep cuts gave a blot of more than 1 cm in diameter after the first half minute It is possible that the bleeding times from cuts deeper than 2 mm will not differ much, but we have not investigated this point

It seems that our 2 mm deep cuts in the ear lobe were comparable to Duke s cuts as judged by the blood lost during the first half minute Nevertheless, Duke found a normal range of *1—3 minutes*, which is far short of our findings in normal persons with cuts 2 mm deep Our range and median for normal persons was also considerably longer than the normal figure given by most other workers Duke did not state whether he held the cutting edge parallel to or perpendicular to the skin and it is also possible that our disposable blades were sharper than his It might well be that the bleeding time is longer from a 2 mm deep cut made with the cutting edge almost parallel to the skin than from a deep and narrow stab with the cutting edge perpendicular to the skin surface When a method is even slightly modified it is clearly necessary to collect a normal material to define the normal values for the particular method

When bleeding time cuts are made

with the edge held parallel to the skin surface, measures should be taken to ensure a correct depth, e g , by measuring with the aid of a hemostat as described above This is especially important in the ear lobe If the cut depth varies considerably, it is not possible to use the bleeding time to evaluate, for example, the effect of plasma transfusions in von Willebrand s disease If the person who makes the cut is not completely unbiased, the cut made after the infusion might unconsciously be made more shallow, with the possibility of seriously misleading conclusions The worker who performs such studies should therefore be unaware of whether the previously injected preparation has been a placebo or the material under test This precaution should be taken no matter how the bleeding time test is performed The bleeding time should of course also be the average of 2 or 3 cuts, as is customary at least with the Ivy technique

Summary

Attention is called to the two different practises of making a bleeding time cut with a Bard Parker no 11 surgical blade If the cutting edge is held perpendicular to the skin surface, the resulting cut is deep and narrow If the cutting edge is held almost parallel to the skin surface the cut tends to be longer and shallower

In the present study all cuts were made with the cutting edge parallel to the skin surface We measured the bleeding time from cuts of different depth, and found that the bleeding time was longer the deeper the cut Measures should therefore be taken to ensure a uniform depth of the cuts A technique for standardization of the depth is described There is a definite danger of unconscious bias when the bleeding time is used to assess the effect of plasma transfusions, e g in von Willebrand s disease Precautions should be taken to avoid this danger

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A Possible Connection Between Carbon Monoxide Exposure, Capillary Filtration Rate and Atherosclerosis

Preliminary report

By

J Siggaard-Andersen, K Kjeldsen, F Bonde Petersen and P Astrup

As recently shown by Astrup et al (1) small amounts of CO in the inspired air increase cholesterol atheromatosis in rabbits fed on a diet containing 2 % cholesterol Filtration of lipid-containing plasma through the vascular wall is considered by many to be the most important factor in the development of atherosclerosis (6)

To examine whether filtration processes can be of significance for the atherogenic effect of CO, studies have been made in human subjects of the magnitude of capillary filtration rate before and after CO inhalation

Methods

The capillary filtration rate and resting blood flow/volume were determined with an air filled rubber cuff plethysmograph (3) by the technique of Celander and Marild (2) The carboxyhaemoglobin saturation was determined by filter photometry as described by Hellung Larsen et al (4)

Experimental procedure

The experimental subjects rested recumbent for 30 min before the measurements The room temperature varied between 72 F — 79° F but was fairly constant during the period covered by the experiment about 2 1/2 hours Venous occlusion was produced by means of a cuff immediately proximal to the upper margin of the patella The cuff of the plethysmograph was placed at the maximum circumference of the calf The change in volume during the second minute of a two-minute period of stasis was taken as an indication of the magnitude of the capillary filtration, which was measured three times at intervals of 5—10 min. with cuff pressures of 40 60 and 70 mm Hg respectively The measurements were repeated after inhalation of 35—50 l of 0 45 % CO in atmospheric air from a Douglas bag giving a CO saturation of the haemoglobin varying between 9 and 16 %

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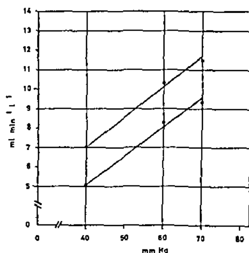


Fig 1 Relationship between filtration rate and cuff pressure Lower curve during breathing of atmospheric air upper curve after carbon monoxide inhalation

Material

Seventeen subjects (12 men, 5 women) were examined Their mean age was 26 ranging from 19 to 51 years

Results

Fig 1 shows the relationship between the magnitude of the filtration rate (ml min^{-1} l^{-1}) and the cuff pressure (mm Hg) The inhalation of the CO is seen to cause a parallel shift upwards At a cuff pressure of 40 mm Hg the change in the magnitude of the filtration rate after exposure to CO was + 1 98 (SD = 1 78), at a cuff pressure of 60 mm Hg, + 2 03 (SD = 1 40), and at a cuff pressure of 70 mm Hg + 2 16 (SD = 1 31) The rise in filtration was significant at all three cuff pressures ($p < 0\ 001$) The resting blood flow/volume before inhalation of CO was 24 2 ml min^{-1} l^{-1} (SD = 10), and after CO inhalation it was 27 6 ml min^{-1} l^{-1} (SD = 10) This difference was significant ($p < 0\ 001$) There was apparently no relationship between the level of the CO saturation and the magnitude of the filtration rate, at the CO saturations obtained here

Discussion

All other factors being equal, capillary filtration rate in normal subjects must vary with the fall in pressure along the capillary, the surface area of the open capillaries and the permeability of the capillary membrane The rise in the resting blood flow/calf volume before and after inhalation of CO suggests that changes in the haemodynamic conditions might contribute to the rise in capillary filtration However, auscultatory measurements of blood pressure and palpation of the radial pulse showed no changes as a result of the CO inhalation Further, the resting blood flow/muscle volume was found unchanged in 9 subjects when measured by the method of Lassen et al (5) depending on Xe^{133} clearance The rise in the resting blood flow/calf volume must therefore have taken place in the non muscular tissue but was in any case too modest (14 %) wholly to explain the rise in filtration, which was 39, 24 and 23 % respectively, at cuff pressures of 40 60 and 70 mm Hg

The rise in the capillary filtration rate is, then, probably partly due to either dilatation of the capillaries, an increase in the number of open capillaries or increased capillary permeability A combination of these factors is also possible

The increased capillary filtration during acute exposure to CO has not been previously demonstrated As mentioned animal experiments with chronic exposure to CO have shown increased lipid deposition in the vessel wall It would seem reasonable to compare these results with those found here The explanation of the atherogenic effect of the CO might perhaps be found in the change in capillary filtration Further studies on this question are in progress

Summary

Inhalation of CO in normal subjects gives a significant increase in capillary filtration A connection between the atherogenic effect of CO and this observation is suggested

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Book reviews

Evolution of the forebrain Phylogenesis and ontogenesis of the forebrain I III Edited by R Hassler and H Stephan 464 pages 295 ill DM 96 — Georg Thieme Verlag Stuttgart 1966

This book of 464 pages contains the 43 papers presented at a symposium on the phylogenesis and ontogenesis of the forebrain at Frankfurt and Sprendlingen in August 1965 On grounds of space the subsequent discussions have not been included

Learned specialists from many countries deal with such different themes as the primordial amygdaloid complex of the African lungfish, brains from 40 million year old fossile camels, electron microscopic cytodifferentiation of embryonal chicken brains The 295 illustrations are of high quality A detailed review is unfortunately impossible on grounds of space

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Symposium International sur la Neuro endocrinologie 354 pages 74 ill L'Expansion, Paris 1966

In this book H P Klotz has edited the papers presented at the 'Journees d'endocrinologie et de nutrition" in Paris from 30 Sept to 1 Oct, 1966 It gives an excellent picture of neuro endocrinology of today The 354 pages contain information about the hypothalamus pituitary system from the histological, biochemical, physiological and even clinical points of view Many chapters thoroughly treat important endocrinological problems such as hypothalamic control of the secretion of ACTH, TSH and gonadotropins, the mechanisms involved in the diurnal rhythm of adrenocortical function, the effect of various hormones on the central nervous system, etc Each chapter is a detailed review of the field and the references are usually very comprehensive (in some instances more than 150)

This book is recommended to all who wish to penetrate the basic mechanisms governing the hypothalamus pituitary system Its contents are valuable to the specialist as well as to those with a general interest in physiology

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The Segmentation of Polymorphonuclear Neutrophils

The conditions in hypovitaminosis B_1 and hypersegmentation

By

EMIL EDWIN

While the shift to the left of the granulocytes has frequently been discussed in haematological literature, less has been written about the opposite process, the shift to the right or hypersegmentation In the literature hypersegmentation is described as a familial anomaly It is also found in neutrophilic leukaemia, erythroleukaemia, chronic nephritis diseases of the liver, cancer and septic diseases (3 9 14 15) It is best known however as one of the morphological changes found in pernicious anaemia and in folic acid deficiency In the latter diseases opinions vary as to the importance of the symptom (13) Its frequency is unknown

There have been few attempts to follow hypersegmentation quantitatively Some of the reasons may be obvious A new counting method must be introduced instead of Schilling s differential count So far there is no agreement as to which of the suggested methods is preferable (2 7 9 11) The definition of hypersegmentation is not clear

The Arneth count is the best known of the different counting methods suggested (2) Arneth recorded the number of lobes in each cell According to this number, five main groups were obtained These were further divided in sub-groups to give a total of twenty This detailed subdivision makes the method complicated and inaccurate

Arneth considers the number of lobes as an expression of the age of the cell This theory could hardly explain the findings in pernicious anaemia, nevertheless it has hitherto been partly accepted

Weicker has pointed out another possibility (16) He maintains that the maturation from promyelocyte to mature polymorphonuclear neutrophil usually corresponds to three cell divisions Should one of these not take place maturation may still be possible but the result will be a hypersegmented cell

By autoradiographic techniques Fliedner et al (8) have been able to demonstrate that the theory of Arneth is probably false The segmentation in granulo

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cytes seems to reflect a still unknown mechanism

In the newer literature two counting methods are employed in clinical work and therefore merit closer discussion

One method is 'lobe average' The lobes in 100 cells are counted, and the total is divided by 100 to give an average value as a measure of the segmentation (9)

Another method consist of counting the cells with five or more lobes Two recent publications may give the impression that this latter method is preferable (4, 10)

Examples in the literature show that counting of hypersegmentation may to some extent give inconstant results (10) One reason may be the error of the counting method Another possibility is that hypersegmentation might be a common sign present in other diseases than those mentioned above

It must be stressed that any differential count may be influenced by suggestibility The counting of hypersegmentation is especially liable to this pitfall Investigations in this field always ought to be done as a blind procedure on coded material

There have now been six aims

1 To find an acceptable definition of hypersegmentation

2 To find a sensitive counting method, minimably influenced by the different technical sources of error such as staining, thickness of smear etc

3 To establish how exact the counting of hypersegmentation is

4 To provide as large a normal material as possible

5 To decide, by comparison of methods in use to day, which one is preferable

6 To ascertain to what extent hypersegmentation occurs in hypovitaminosis B_{12}

Material and methods

The normal material consists of 36 blood donors and 11 patients admitted to the surgical department with minor fractures hernias or varices (27 women and 20 men) The age ranges from 16 to 79 years 25 being under and 22 over 50 years

To test the accuracy of the countings ten consecutive blood smears were taken from a patient chosen at random Representing smears from blood with constant properties they are later in this article referred to as standard smears

Besides the above mentioned smears smears were counted from 35 patients suffering from rheumatoid arthritis and from 16 patients with rheumatoid spondylitis

Blood smears were taken in the ordinary way To obtain a fairly even blood film a square 16 × 16 mm from the thinnest part of each smear was marked off The counting was performed within this square 100 × 5 granulocytes being counted They were allocated among five main groups corresponding to cells with one two three, four or (group 5) five or more lobes

Acceptance of two parts of a nucleus as independent lobes required that the bridge between them should not exceed 50 % of the largest diameter Sex chromatin was not counted when this was reckoned to be present No lower limit, however has been put on the size of the lobes

All smears were coded with random numbers varying from 8 to 1 500 and afterwards mixed to assure a blind test

Counting results from 25 patients with serum B_{12} less than 150 pg/ml have been used to test the different methods and findings These value have been obtained from a previous investigation (6) Over six months counting was done in smears from

all patients in whom serum B_{12} analysis was due to be performed. The examination was always done before the serum B_{12} level was known. At this time neither lobe average nor segmentation index were known to us. Definitions and counting methods were similar to those in the present paper, but only 200 granulocytes were counted.

All smears were stained with May Grunewald Giemsa staining solution.

The greatest possible light intensity and 1 000 × enlargement were used on the microscope.

Results

The results for the ten standard smears from a single patient have an immediate interest. Here the composition of the blood is kept constant, but the smears will vary in technical standard. On theoretical grounds there will always be a certain degree of dispersion, which may be calculated (12). Should our results substantially exceed these theoretical limits, this is probably caused by technical errors such as subjective evaluation, varying staining technique, varying film thickness, overlapping of lobes etc.

Table I gives the counting results for these ten standard smears. On comparing the observed variation with that theoretically expected, the correspondence is good in groups 1, 3 and 5, and poorest in those with two or four lobes.

We may postulate that the precision of a method of registration depends on two factors:

1. The minimum variation in ten standard smears as mentioned above.

2. The maximum variation in a patient material.

Lobe average seems promising in respect of the first requirement.

Table II shows the results in our normal material. To our knowledge, this is the largest normal material published. Like others, it has the weakness that the grouping of a cell is a matter of subjective evaluation. The figures, however, tally well with those from some other investigations (14).

No significant age difference has been found. Some sex difference is found in group 5: women 2.36 %, SD 2.08; men 2.02 %, SD 2.17.

This difference is not statistically significant, but is mentioned because previously we found a similar tendency (5). It is possible that a larger material might give a significant difference.

In table II several alternative expressions are given for the normal range of variation. In most cases ± 2 SDs match well the observed values. Lobe average forms an exception. A closer examination reveals a skew distribution especially for lobe average but also to some extent for group 5. On this basis we find it correct to use 95 % of the observed dispersion as normal range of variation. The values are given in the last row of table II.

To find an acceptable definition of hypersegmentation is difficult. We have studied the relations between cell groups. A symbolic presentation of the different graphs is found in table III. The key to interpretation is as follows.

Our starting point has been that the cells with five or more lobes must be found more often in patients with hypersegmentation. On examining how the cell group with one lobe varies with in-

TABLE I Counts obtained from a single patient Ten blood smears taken simultaneously

	1 segm	2 segm	3 segm
Mean	1 0 %	14 1 %	49 9 %
S D	1 18	7 02	4 78
Coefficient of variation	118 %	49 7 %	9 6 %
Variation observed	0—3 1 %	5 5—26 1 %	45 5—55 3 %
Variation theoretically expected	0—3 0 %	11 0—18 0 %	45 0—55 0 %

TABLE II Normal values for a control group Thirty six blood donors and 11 patients with minor

	1 segm	2 segm	3 segm
Mean	2 1 %	19 2 %	49 1 %
S D	2 36	9 35	4 89
Mean ± 2 S D	0—6 8 %	0 5—37 9 %	39 3—58 9 %
Variation observed	0—11 5 %	6 4—40 1 %	40 2 —57 4 %
Normal range 95 % of obs values	0 2—7 7 %	7 1—39 8 %	40 9—56 5 %

TABLE III Correlation between different groups of cells

	1s -5s	2s -5s	3s -5s[1]	4s -5s	1s -2s	4s -L a	2s -L a
GRAPH (symbolized) (1st group as ordinate)							
Sign of correlation	Neg	Neg	Pos/Neg	Pos	Pos	Pos	Neg
Coefficient of correlation		-0 72		+0 79	+0 83	+0 97	0 96
Coefficient of regression		-0 17		+0 16	+0 21	+0 026	-0 029

[1] 4 or 2 segmented groups give similar results

4 segm	5 segm or more	Lobe average	Segm index
30 6 %	4 4 %	3 23	13 9 %
8 94	2 02	0 215	3 19
29 6 %	45 8 %	6 7 %	22 9 %
17 5—45 8 %	2 1—7 5 %	2 90—3 54	9 2—19 3 %
26 5—35 6 %	2 1—7 1 %		

fractures hernias or varices

4 segm	5 segm or more	Lobe average	Segm index
27 0 %	2 6 %	3 08	8 5 %
10 80	2 17	0 285	5 11
5 4—48 6 %	0—6 9 %	2 51—3 65	0—18 7 %
6 2—47 6 %	0—7 1 %	2 40—3 52	0—18 5 %
9 3—45 1 %	0—6 8 %	2 44—3 49	0—16 9 %

creasing values of group 5 one finds a negative correlation i e that the cell with one nucleus is atypical in hyper segmentation The same argument applies to cells with two lobes Cells with three lobes have an intermediate position no linear graph can be drawn for the relation between group 5 and group 3 Cells in groups 4 and 5 are positively correlated Either may be taken as expression of a shift to the right

In table III another condition of interest is found In the two last columns the relation is given between lobe average and number of cells in groups 4 and 2 One notices the high correlation coefficients +0 97 and —0 96 The probable conclusion is that lobe average is mainly an exponent of the relation between these two cell groups Returning to table I one sees that the same cell groups have a large counting error Table II shows a considerable degree of normal variation in the two groups

Segmentation index

One aim of this investigation was to find a sensitive method which compared with earlier methods would be less affected by the different sources of error We thought these requirements might conceivably be met if hypersegmentation

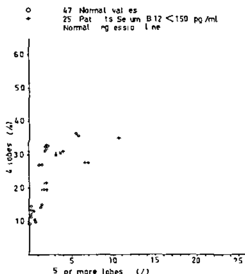


Fig 1 The diagram demonstrates how a disproportionate increase in the number of cells *with five or more lobes is a typical feature* of hypersegmentation

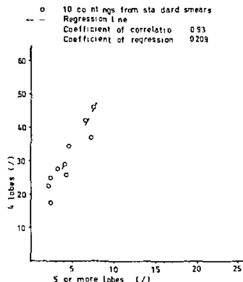


Fig 2 The diagram depicts how the 4 segm 5 segm ratio is a relatively invariable *value compared with the values for groups 4* and 5

could be expressed by a ratio between two cell groups The cell groups ought morphologically to be so closely related that staining technique and thickness of smears would influence the two groups in the same way, the ratio thereby remaining constant

To ascertain whether such a characteristic ratio exists, 25 patients with serum B_1 below 150 pg/ml have been examined This group of patients should presumably show hypersegmentation By plotting these results into the graphs of normal correlations (symbolically shown in table III) the eventual characteristics of hypersegmentation should appear As a result of this analysis it was found that the relations among the groups 1 2 3 4 were essentially unchanged in the B_1 group What seemed typical was a disproportionately large increase in the number of cells with five or more lobes This we think, may be an important point in the definition of hypersegmentation

The disproportionate increase in cells with five or more lobes is best seen by comparing group 5 with group 4 This is shown graphically in fig 1 We find that the distance from the normal regression line in the figure seems to be a good expression of the hypersegmentation in our B_{1-} group

There remains the problem whether groups 4 and 5 are influenced in the same way by different technical errors

A hint of relevance may be found in our ten standard smears Here the composition of the blood is constant, but the ten smears do vary in staining thickness etc In smears of high quality, one expects to find complicity such that many cells are in groups 4 and 5 De

creasing quality will give a lower enumeration of multi lobed cells

The results in our standard smears are graphically demonstrated in fig 2 In spite of a relatively high dispersion of the counting results in groups 4 and 5, the observations made deviate very little from the regression line The coefficient of correlation is as high as + 0,93 In these ten smears the requirement that group 4 and group 5 ought to be influenced in the same way by different staining technique is more than fulfilled

The results have led us to establish the following formula

Segmentation index =

$$\frac{\text{granulocytes with 5 lobes or more} \times 100}{\text{granulocytes with 4 lobes}}$$

This segmentation index is an approximate expression of the principles mentioned above and is calculated in all the tables It has a relatively low coefficient of variation in the standard smears

To investigate which method would be the best for practical use we have compared the coefficient of variation in the standard smears with that in the combined material of blood donors patients with polyarthritis and spondylarthritis

Table IV shows how groups 1 and 2 have coefficients of variation as high in the standard smears as in the total material These cell groups are thus unsuitable for the evaluation of hypersegmentation Group 3 also shows a minimal difference and must be ruled out

Group 4 group 5 lobe average and segmentation index remain to be considered as theoretically possible measures of hypersegmentation

The difference in coefficient of variation between the standard smears and the total material can be expressed as a percentage This is done in the last row of table IV From these figures it may directly be seen that the segmentation index must be reckoned the most sensitive method

It remains to test the different methods in practice To do this, we have used our normal values (last row table II) to show how many of the patients with hypovitaminosis B_{12} reveal hypersegmentation by the different methods

Table V gives a comparison among the three potential methods It appears that relatively few patients have hypersegmentation as judged by lobe average From number of cells with five more lobes, diagnosis can be made in 44 % of the patients, a rather disappointing result However, the figure agrees well with results found earlier in a subjectively evaluated material (5) Others have made similar findings when evaluating folic acid deficiency from group 5 (10)

As expected the segmentation index is a more sensitive method By this method, 64 % of the patients with serum B_{12} less than 150 pg ml show hypersegmentation

The group 5 method is not significantly better than the lobe average $p = 0{,}06$

The difference between the segmentation index and lobe average is highly signicant $p = 0\,001$5

Discussion

The investigation has shown a considerable counting error The counting results for ten blood smears from a single patient

TABLE IV Comparison of the coefficients of variation (%) A The total material (98 counts)

	1 segm	2 segm	3 segm
A Coefficient of variation Total material	105 6	46 0	11 2
B Coefficient of variation Standard smear	118 0	49 7	9 6
Difference (A—B)			1 6
Difference % (A—B) 100 / A			14

TABLE V Evaluation of hypersegmentation by different methods Twenty five patients with serum B_{12} below 150 pg ml

Method	Hyper segmented	Normal segmented
Lobe average	4 pats (16 %)	21 pats (84 %)
Number of cells with 5 lobes (or more)	11 pats (44 %)	14 pats (56 %)
Segmentation index	16 pats (64 %)	9 pats (36 %)

call for caution when evaluating a single counting result in a patient Having examined 70 000 cells one wishes to stress that in investigations of this kind it is a prerequisite that the material be carefully coded and examined by a blind trial

Our investigations do not support the theory of Arneth that segmentation is an expression of the age of the cell If this were true one would expect the same relationship between groups 4 and 5 in a normal material as in a group of patients with hypovitaminosis B_{12} The finding of a disproportionate increase of cells in group 5 is more easily explained by Weicker s theory of the maturation sequence of the neutrophils

What we have called the segmentation index seems to be the most sensitive method and is also reckoned to be less influenced by technical errors etc This is not a postulate founded on theoretical considerations alone but is supported by the variation coefficients of the standard smears (table I), and by the end results (table V)

There has been debate whether morphological changes in haematopoiesis are always *present in B_{12} deficiency* or whether we have to rely on biochemistry This question cannot easily be answered One of the signs hypersegmentation may be of some restricted value An important point is however that hypersegmentation appears in diseases other than B_{12} and folic acid deficiency Some of these diseases have been described in the literature, but the author has reason to believe that the

B The standard blood smear (ten blood smears from a single patient)

4 segm	5 segm	Lobe average	Segm index
38 4	94 5	9 2	71 1
29 6	45 8	6 7	22 9
8 8	48 7	2 5	48 2
23	52	27	68

symptom may also be found in other groups of diseases

Conclusions

1 Cells with one or two lobes are considered to be measures of *shift to the left* Cells with three lobes have an intermediate position Cells with four or more lobes are measures of shift to the right

When cells with five or more lobes are found disproportionally often this seems to be characteristic for hypersegmentation

2 The ratio between cells with five lobes and cells with four lobes expressed as percentage is a useful working method This ratio is called segmentation index

3 The counting error in counting lobes exceeds to some extent theoretically calculated dispersion This applies especially to cells with two or four lobes

4 Values within 95 % of observed dispersion can be taken as normal

5 Lobe average is the least sensitive of the methods in use Counting of cells *with five or more lobes probably comes* next Segmentation index is the most sensitive method

6 Estimates of hypersegmentation in hypovitaminosis B_{12} will vary according *to the method used in evaluation With* the segmentation index, hypersegmentation is found in 64 % of the cases

Summary

The author has examined 47 normal persons with regard to the segmentation of the granulocytes Normal values for the different cell groups are given The counting error has been evaluated An *analysis has been made of the behaviour* of cells under normal conditions and with a shift to the right

A new method for the evaluation of hypersegmentation is suggested A theoretical *appraisal and a practical test of* the efficiencies of the different methods have been made

In a group of patients with vitamin B_{12} deficiency the new method gives 64 % hypersegmentation

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Less Common Manifestations of Hyperparathyroidism

By

Lennart Andersson and Tore Lindholm

After more than 30 years study of hyper parathyroidism Hellstrom (13) stated in his last paper on the subject The symp toms in hyperparathyroidi m are so numerous and non characteristically varied that a test of the serum calcium should be a routine examination at all clinical examinations, just as the tests on albumin in the urine

During investigation of 138 cases of primary hyperparathyroidism Hellstrom and Ivemark (14) found nephrocalcino-sis or urinary lithiasis in 86 %, and roentgenological evidence of skeletal in volvement in 44 % Since these are the more usual manifestations of tne disease, most patients with parathyroid hyper function are referred to surgical or uro-logical departments However, in a number of cases symptoms mainly relate to other systems e g the gastro-intestinal tract (11) or pancreas (4 7 15 24) Hyperthyroidism (10 29) and mental changes (8 28) have also been reported in association with hyperparathyroidism

During a short period we have ob-served five patients whose initial symp toms produced no suspicion of para thyroid disease These cases are a good illustration of the varied clinical presen tation of the syndrome

Submitted for publication February 27 1967

Case reports

Case 1

A woman born in 1919 had a history of chronic pyelonephritis since the age of 21 Medical examination in 1946 showed a slightly contracted left kidney but no evi dence of calculi Further examination two years later showed a reduction in the kid ney size and clubbing of the calyces on both sides Kidney function was reduced and the non protein nitrogen was 51 mg per 100 ml Subsequently kidney function gradually deteriorated and in 1961 the non protein nitrogen rose to 100 mg/100 ml The patient complained of pain in the back thighs elbows and fingertips Roentgenological examination showed a general osteoporosis with subperiosteal re orption and destruc tion of the sacro iliac joints In addition there was resorption of the lamina dura around the teeth All these changes pointed to a diagnosis of hyperparathyroidism The

serum calcium was normal and the serum inorganic phosphorus raised The condition was regarded as one of hyperparathyroidism secondary to chronic kidney disease and treatment with AT 10 (dihydrotachysterol) was *instituted As a result of this treatment* the skeletal pains improved as did the roentgenological picture of the bones During the following years serum calcium was kept between 4 8 and 6 1 mEq/l Uraemic symptoms started in 1962 and were dominated by pruritus and an anaemic tendency requiring repeated blood transfusions The patient had a considerable salt loss which was treated with sodium chloride 10—12 g daily In the summer of 1966 her condition worsened with increasing tiredness pruritus and a constant dry cough She developed multiple fractures of the ribs and the right clavicle

In September 1966 the patient started a twice weekly programme of haemodialysis with the artificial kidney Her condition improved considerably but the dry cough *and bone pains remained In an effort to* improve the skeletal lesions and in preparation for a future kidney transplantation it was decided to resect the parathyroids At operation on January 23 1967 a left sided parathyroid adenoma was found 36 × 20 × 15 mm in size as opposed to the expected diffuse hyperplasia The adenoma was removed The postoperative period was uneventful So far there has been no improvement of the kidney function and haemodialysis has been continued Within a few days of operation there was considerable improvement of her skeletal pains The distressing dry cough which had persisted for more than half a year disappeared immediately after operation

Case 2

A man born in 1912 had suffered from a duodenal ulcer in September 1966 and many years previously On several other occasions he had complained of pain in the upper abdomen nausea and vomiting On December 5 1966 he had developed sudden intense epigastric pain radiating to both sides together with vomiting On admission abdominal tenderness and rigidity were found most marked in the epigastrium Intravenous fluid therapy was instituted In spite of the administration of mannitol and *Lasix®, anuria developed Ureteric catheteri*zation excluded a postrenal obstruction Explorative laparotomy showed an acute pancreatitis with wide spread fat necrosis The abdomen was therefore closed Since the anuria continued, peritoneal dialysis was commenced on December 8 This had the double purpose of correcting the uraemia and if possible of removing pancreatic enzymes and break down products Immediately after laparotomy an intravenous injection of a protease inhibitor (Trasylol Bayer) was given This drug was also added to the peritoneal irrigation fluid (50 000 I U /1 000 ml) The patient s general condition improved, and urine production recommenced during the dialysis which continued for five days The serum calcium initially 6 8 mEq/l remained at a raised *level 5 1—5 8 mEq/l throughout* treatment in spite of the active pancreatitis The failure of the serum calcium to fall during dialysis may be explained by the presence of calcium 3 5 mEq/l in the irrigation fluid

During the subsequent few days the patient s condition deteriorated with increasing abdominal pain uraemia and falling serum calcium Peritoneal dialysis was therefore restarted on December 17 and continued for five days His condition improved again the abdominal pain disappeared and he gradually became mobilised

However his renal function slowly decreased The blood urea nitrogen increased from 70 to about 200 mg/100 ml over a period of a few months The serum calcium varied between 4 1 and 6 8 mEq/l His *appetite was poor together with occasional* vomiting and a resulting weight loss Trypsin excretion was normal but the glucose tolerance test showed a slight diabetic tendency Insulin therapy produced no improvement in his appetite Roentgenological examination of the stomach and

gall bladder were normal Except for a small cavity in his right fibula roentgenological examination of the bones was normal A straight X ray of the abdomen showed scattered calcification of both kidneys which were of normal size Retrograde left-sided pyelography showed a markedly deformed pelvis with widespread papillary destruction The combination of peptic ulcer pancreatitis hypercalcaemia kidney and skeletal changes suggested a diagnosis of hyperparathyroidism At operation a parathyroid adenoma 15 × 8 × 7 mm in size was found and removed Postoperatively there was a marked fall in serum calcium to 3 mEq/l a level which could only be maintained by vitamin D and large doses of calcium. Within a few days the patient who had previously been somewhat withdrawn and negative became lively and of extrovert character

Case 3

A man born in 1898 had symptoms of hypertension since 1950 In 1964 he had left sided loin pain produced by a stone in the left kidney The stone was removed On 26 October 1965 he developed a left sided hemiparesis, which gradually subsided during the following week He was referred to the neurological department for further investigation

Examination showed a tired and uncooperative man with a blood pressure of 200 115 mm Hg He had a moderate central facial paresis but no peripheral motor disturbances His left arm and leg showed increased reflexes His Babinski was negative He had a grade II—III *hypertensive* retinopathy Cerebral angiography was normal except for a slight narrowing at the bifurcation of both common carotid arteries Kidney function was moderately reduced creatinine clearance was 66 ml/min specific gravity 1 015 Serum calcium was raised to 5 6—6 0 mEq/l, and serum inorganic phosphorus slightly low 1 8—2 3 mg/100 ml Roentgenological examination showed no nephrocalcinosis, urinary or skeletal changes

During his period in hospital he became increasingly tired irritable and confused He was difficult to rouse and had marked constipation

Since hyperparathyroidism was suspected the patient was transferred to the Urology Unit on December 8 Operation was performed A parathyroid adenoma 20 × 10 × 10 mm in size was found behind the lower pole of the right thyroid lobe and removed Simultaneous examination of the carotid arteries showed no abnormality

Within a few hours of operation the calcium level fell and was normal after 24 hours 4 2 mEq/l It remained at this level His mental condition deteriorated, he became aggressive and violent, requiring large doses of sedatives He was given vitamin D and calcium orally After two weeks his mental symptoms regressed but his hypertension remained unchanged He was no longer troubled by constipation

Case 4

A woman born in 1901 had oil collapse therapy for pulmonary tuberculosis in 1933 The infection became active again in 1954 Following antibiotic therapy she was considered cured in 1961 In July 1966 she developed a productive cough, pyrexia and tiredness and became practically bedridden. Examination showed penetration of the oil into the adjacent lung A bronchial fistula was suspected, and the patient was referred to the thoracic department

At this time she was mentally normal Two months later she seemed mentally changed but psychiatric investigation was *inconclusive* Laboratory *investigation* showed a raised serum calcium 8 2—8 6 mEq/l but a normal serum inorganic phosphorus

In view of the high calcium level dialysis was considered and she was referred to the Renal Clinic She appeared disorientated apathetic and somewhat unconcerned about her condition She was unable to read or understand a daily newspaper

Since no other cause for her hypercalcaemia was found hyperparathyroidism was

suspected kidney function was reduced, with an endogenous creatinine clearance of 27 ml/min Roentgenological examination showed no calcification in the urinary tract At operation she was found to have a parathyroid adenoma 15 × 10 × 10 mm in size behind the left thyroid lobe The serum calcium fell slowly to normal 4 4 mEq/l 7 days after the operation No immediate improvement of her mental condition was noted On the 2nd and 3rd postoperative days she was depressed but became aware of her condition After a further few days she brightened mentally and started to cooperate In the subsequent weeks her condition continued to improve but some mental abnormality remained

Case 5

A woman born in 1929 sought medical advice in June 1966 because of headache and vomiting occurring in connection with menstruation Examination showed a left sided neck swelling which was thought to be a goitre She was re examined on July 15 following a three day episode of vomiting Her mental state was normal Her vomiting was temporarily relieved with promethazin chloride Lergigan®) but on the following evening July 17 she became sleepy and confused and this was thought to be due to the promethazin The following morning the patient became hypotonic and delirious Her B P was 85/60 and pulse 110 There were no localized neurological signs She was referred to hospital with a suspicion of a fluid and electrolyte disturbance On admission laboratory investigation showed sodium 150 potassium 3 2 calcium 12 2 mEq/l standard bicarbonate 25 mM/l serum inorganic phosphorus 4 0 mg per 100 ml and non protein nitrogen 142 mg per 100 ml

The patient was immediately referred to the renal department On admission she was stuporous reacting to pain and noise but no mental contact could be established The peripheral reflexes were increased the plantar response was normal Her pupils were of equal size and reacted equally to light Surgical and gynecological examination was carried out because of her previous abdominal pain but the results were inconclusive

The patient was considered to be in a state of acute hyperparathyroidism Because of her poor condition it was considered desirable to correct her profound fluid electrolyte disturbance before operation She was given 3 000 ml of invertose with potassium chloride and haemodialysis was performed The patient s electrolyte situation improved and serum calcium fell from 10 8 to 5 9 mEq/l

Operation was performed three hours after dialysis The swelling in the neck was found to be a large parathyroid adenoma, 30 × 20 × 15 mm lying in the thoracic inlet and extending into the mediastinum After removal of the adenoma exploration of the other glands showed no additional tumours

During the first 24 hours after operation the serum calcium remained at about 6 mEq/l but after a further 12 hours it fell to 4 7 and remained at this level The patient became mentally clear within 12 hours of the operation and was able to hold a normal conversation She soon became mobilised She was given oral vitamin D and calcium and in the first week, intramuscular prednisolone

Repeated measurements of serum calcium and inorganic phosphorus after the operation have been normal and kidney function had returned to normal after three months with urine specific gravity 1 026

Discussion

Case 1 was the only patient, in whom kidney disease dominated the clinical picture from the beginning She had a 25 year history characteristic of chronic pyelonephritis with episodes of acute in

fection The nephropathy described in primary hyperparathyroidism consists of a calcium deposition and destruction of the tubular cells (5 34), which may produce a radiological picture of nephro calcinosis (14) However an advanced hypercalcaemic nephropathy may exist without any radiologically detectable changes (20) Even though *case 1* had chronic pyelonephritis, the coexisting hypercalcaemia of long duration proba bly accelerated the progression of kidney destruction It is well known that chronic kidney di ease can produce parathyroid hyperfunction and a generalized hyper plasia of the parathyroid glands (26) The discovery in this case of a solitary parathyroid adenoma indicates that it was primary hyperparathyroidism Since it is difficult to distinguish cases of primary and secondary hyperpara thyroidism and since the former can be radically treated by operation it is ad visable to regard all cases as primary un til proved otherwise

Even in *case 2* there was a progressive advanced nephropathy with widespread papillary destruction and kidney calcifi cation However, the patient presented as a case of acute pancreatitis and not as one of renal disease A relationship be tween hyperparathyroidism and acute pancreatitis was first described by Cope et al (4) who stated that pancreatitis may produce a fall in the calcium level thus making hyperparathyroidism diffi cult to detect In the case described here however the calcium level re mained raised during the acute episode Several cases of acute pancreatitis in as sociation with hyperparathyroidism have been described (7 15, 24, 30, 33) but the relationship between these two con ditions is not clear Cope et al (4) and Hoar and Gorlin (16) considered the pancreatitis to be due to precipitation of calcium in the pancreatic ducts Other writers (14) have been unable to find any pancreatic calculi at autopsy Hueper (18) and McJunkin et al (19) found in animals that excessive doses of para thyroid hormone may produce pan creatic necrosis suggesting a primary role of the parathyroid hormone itself Although the etiological connection is not clear clinical evidence strongly upports the correlation between acute pancreatitis and hyperparathyroidism Therefore serum calcium estimations should be carried out in all cases of acute pancreatitis both during the acute phase and on recovery

Case 2 had both acute necrotising pancreatitis and acute renal failure In our experience (1) this combination carries a very poor prognosis, and all previous cases have been fatal The pa tient s condition improved with peri toneal dialysis but deteriorated again when treatment was discontinued Sub sequent peritoneal dialysis again pro duced an improvement Peritoneal dialy sis therefore probably contributed to the succe sful outcome The aim of the dialy sis was to reduce the uremia and to eliminate the toxic break down products from the pancreas

In addition this patient had at least two previous episodes of duodenal ulceration Peptic ulcer is the most common gastro intestinal manifestation of hyperpara thyroidism (3 11 12) Ostrow et al (25) found that in several large series of cases with hyperparathyroidism the

frequency of ulcers was 9 1 % The association between peptic ulcer and parathyroid hyperfunction is not established in the majority of cases In a smaller number of cases parathyroid adenoma has been found together with adenomas in other endocrine organs, e g the pancreas (9, 31, 32) It is remarkable that in some cases described, a chronic ulcer, resistent to treatment, has healed following removal of the parathyroid adenoma Other gastro intestinal symptoms include nausea and vomiting, marked anorexia, epigastric distress, diarrhoea or constipation *Case 5* had severe vomiting and diarrhoea, while *case 3* complained of constipation, which was relieved by removal of the tumour Usually these gastro intestinal symptoms are too vague to establish a definite diagnosis but they are an important feature of the clinical picture

Two of our patients *(cases 3 and 4)* had marked mental symptoms, leading to immobilisation and confusion *Case 4* was completely disorientated Although operation was followed by a considerable improvement in both cases, there was some residual mental change Many writers (21 33 and others) have drawn attention to the occurrence of mental symptoms in hyperparathyroid patients usually lethargy drowsiness, confusion and depression Generally these symptoms occur in combination with disturbances of other systems but sometimes as in the cases reported here they dominate the clinical picture (8 28)

Severe cerebral disturbances stupor and coma occur especially with extremely high serum calcium levels, such as those found in acute parathyroid crisis (6) In this condition severe symptoms develop in various systems, as in *case 5*, who had vomiting, diarrhoea and lethargy, leading to a state of coma This condition is rapidly fatal if untreated In a survey of 70 cases of parathyroid crisis Payne and Fitchett (27) reported a mortality of 59 % This condition is both a medical and surgical emergency The correct treatment is immediate surgery with removal of the adenoma or hyperplastic glands, but these patients have a severe fluid electrolyte imbalance, which increases the operative risk It is therefore advisable to correct this imbalance before commencing surgery With the use of dialysis we have been able to reduce a markedly elevated serum calcium This is in agreement with the findings of Antila et al (2) and Maxwell et al (22) However, the benefit of dialysis is of short duration (23) and operation should be attempted as soon as possible It should be noted that choline esterase levels are low after dialysis (17) and therefore succinyl choline preparations should not be used during anesthesia

Summary

Five cases with less common manifestations of hyperparathyroidism are described In one patient the parathyroid hyperfunction was thought to be secondary to chronic pyelonephritis, but an adenoma was found at operation Another patient had recurring duodenal ulceration and acute necrotising pancreatitis He was treated with peritoneal dialysis and later parathyroidectomy

Mental symptoms of tiredness and con fts on dom nated the cl nical picture in t o cases They improved but did not completely disappear after operation The 5th patient had an acute para thyroid crisis with severe hypercalcaemia dehydration and uraemia After initial correction of the electrolyte imbalance using haemod alysis a parathyroid ade noma was removed All patients survived

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Optic Atrophy and Juvenile Diabetes Mellitus with Familial Occurrence

By

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In the early literature on diabetes mellitus, optic atrophy was occasionally mentioned as a possible complication (8) In 1935 Waite and Beetham (19) concluded from a statistical study that the incidence of optic atrophy in a general material of diabetic patients was not significantly higher than in a control group of non diabetics In 1938 Wolfram (20), however reported a family in which diabetes mellitus and optic atrophy were simultaneously present in four out of eight siblings Since then another eleven (1 3, 5 6, 9, 10 11 12 15 17, 18) families have been reported presenting this combination in althogether 27 cases generally with *sibling* relationships Exceptionally in one of the families (11) the disease occurred not in siblings but in a woman and in a daughter of her healthy sister This family is however specially interesting as being the only one in which the disease occurred in two generations

The literature up to 1965 was recently reviewed and analyzed by Rose et al together with seven casuistic observations of their own (9) Only two of their own cases were siblings the others being unrelated These authors draw a conclusion already suggested by Tyrer in 1943 (18) that the coincidence of diabetes mellitus and optic atrophy is the expression of a specific genetic abnormality

Optic atrophy and diabetes mellitus thus seem to be a specific constellation *sui generis* and as such of considerable theoretical and practical interest Probably it is still sometimes overlooked Accordingly, we feel it warranted to report another family with this interesting syndrome *at present under observation in* this hospital

Case reports

Our own cases belong to a series of five siblings of whom only the eldest sister is healthy in all respects The four younger siblings (one brother our original case and three sisters) all have diabetes mellitus and bilateral optic atrophy (fig 1)

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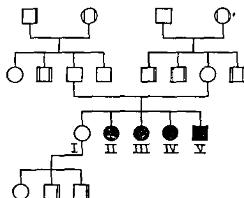


Fig 1 Pedigree of the present family □ = normale male ○ = normale female Black symbol patient with bilateral optic atrophy and juvenile diabetes mellitus Vertical line in the left half of the symbol relative not known to have diabetes but not specially examined Vertical line in the right half of the symbol relative not known to have optic atrophy but not specially examined

A The Siblings

Case 1 (sibling I)

This was the youngest of the siblings, a male now aged 18 Normal birth and early childhood At the age of 14 he complained of failing vision on examination an unexplained reduction of the visual acuity was found in both eyes (corrected V A 0 7) The presence of an undulating nystagmus was noted but nothing abnormal was found on the fundi His colour vision seems not to have been tested

At the age of 17 he developed consistent glycosuria during a period of fatigue and slight weight reduction His parents with the experience from the three elder daughters were in fact expecting the patient s diabetes to appear at this age He was then admitted to this hospital

On general physical examination he seemed a healthy and well developed youth nothing abnormal being demonstrated Neurologic examination was normal except for the ophthalmologic findings Roentgenologic examinations of the cranium including the optic foramina, and of the lungs were normal The cerebrospinal fluid was normal (including electrophoresis) Audiometry was normal A study of the chromosomes of the lymphocytes showed a normal male pattern There was laboratory evidence of diabetes mellitus with constant hyperglycaemia

On ophthalmologic examination the corrected V A was found to be 0.5 in both eyes The undulating nystagmus was still present The colour vision tested by the method of Ishihara was defective Examination with an anomaloscope revealed a colour pattern of a type seen in acquired defects The media were clear The optic discs were pale and well demarcated There was a sparse pigmentation of the retina but in the periphery a few discrete accumulations of pigment were scattered diffusely A fine granular pigment dispersion was seen at the maculae and there was an indistinct reflex The visual fields were normal Dark adaptation tested by the method of Krakau and Öhman (7) was slightly reduced When on diet the patient had no glycosuria but a slight hyperglycaemia (140 mg %) Tolbutamide had no influence on the blood sugar level which however was fully normalized with a dose of 16 units of soluble insulin a day The patient later refused insulin therapy on diet therapy alone the fasting blood sugar is about 140 mg % but there is no glycosuria

Laboratory findings

Hb white blood cell count and differential ESR serum proteins blood urea urine microscopy serum vitamin B_{12} level and the Wassermann reaction were normal The insulin tolerance test was normal as were cortisol somatotropin, free fatty acids in plasma and serum cholesterol Blood group O C+ D+ E— c—, K— MN ss Le^a+ LE^b—, Fy^a+

Case 2 (sibling IV)

Women age at present 21 Normal birth and early childhood At 18 diabetes mellitu was diagnosed and she was admitted to this hospital

General physical examination revealed

nothing abnormal The V.A was normal and the optic fundi seemed normal The diabetes was and has remained well controlled on diet and 16 units of NPH insulin One year later pale optic discs and a slightly defective colour vision were detected but the V A was still normal

After the detection of the disease in case 1, she was re examined at the age of 20 General physical examination was still normal No neurologic abnormality could be demonstrated Audiometry was normal

In a full ophthalmologic check up the corrected V.A was found to be 0 7 in both eyes the colour vision was now clearly defective (Ishihara) and examination with an anomaloscope revealed a colour pattern of a type seen in acquired defects No nystagmu Clear media The optic discs were pale and well demarcated The vessels were normal There was a sparse pigmentation of the central retina but in the periphery a few accumulations of diffusely scattered pigment were noticed There was a fine granular dispersion of pigment at the maculae and the macular reflex was indistinct The visual fields were normal Dark adaptation (7) was lightly reduced

Laboratory findings

ESR and Hb were normal There was no proteinuria Blood group B C+ D+ c+ e+ K— MM Ss Le^a— Fy^a—

Case 3 (sibling III)

Woman, at present 24 years old Normal birth and early childhood At 14 diabetes mellitus was diagnosed The optic fundi had then a normal appearance and the V A was normal The diabetes was well controlled on diet and 8 units of lente insulin during the first five years but since the age of 20 the control has been less satisfactory

After the discovery of the syndrome in cases 1 and 2 she was re-examined at the age of 24 She was found to be slightly obese but otherways general physical and neurological examinations showed nothing abnormal Audiometry was normal The requirement for insulin was then 24 units The fasting blood sugar was 188 mg % and there was a daily glycosuria of 14 g There was no ketonuria A study of the chromosomes in the lymphocytes showed a normal female pattern.

On ophthalmologic examination the corrected V A was found to be 0 2 in the right eye and 0 6 in the left There was no nystagmus The media were clear There was an advanced retinopathy in both eyes with many haemorrhages and hard as well as cotton wool exudates The latter were arranged in an incomplete macular star A marked macular oedema was found bilaterally The vessels were more tortuous than normal Both optic discs were pale and the disc margins were sharply demarcated Also in this case there was a sparse pigmentation of the retina with some scattered accumulations of pigment in the periphery Equally there was a fine granular pigment dispersion at the maculae and an indistinct macular reflex There was a clearly defective colour vision (Ishihara) The visual fields were normal

Laboratory findings

Normal Hb No proteinuria Normal blood urea Blood group B C+ E— K— P+ MM Ss Le^a— Le^b+ Fy^a+

Case 4 (sibling II)

Woman at present 30 year old Normal birth and early childhood At 16 diabetes mellitus was diagnosed The V.A and the optic fundi were recorded as normal The diabetes was well controlled by diet and 20 units of lente insulin The control was satisfactory for several years but since the age of 25 there has been a poor control partly because of the patient s refusal to be on diet

After the demonstration of the disease in the siblings the patient was re examined at the age of 30 General physical and neurological examination showed nothing abnormal There was no ketonuria and the fasting blood sugar value was 244 mg %

On ophthalmologic examination the corrected V A was found to be reduced on the right eye to 0 2 and on the left eye to

0 3 In both eyes there were a posterior cortical cataract of moderate degree and optic atrophy with white and sharply demarcated discs In the right eye some hard exudates were found as well as a couple of micro aneurysms and a small haemorrhage Also in the left eye there were some retinal exudates Colour vision (Ishihara) was defective The visual fields showed a small central scotoma in both eyes

Laboratory findings
Blood groups B C+ D+ c+ e+ K— NN ss Le^a— Le^b+ Fy^a+

Sibling I
The healthy sister was examined at the age of 33 She had no glycosuria and a normal glucose tolerance test She had normal V A and the optic fundi had a normal appearance Blood group B C+ D+ E+ c+ e+ K— MN Ss Le^a— Le^b— Fy^a+ She had three children 1—5 year old None of them had glycosuria The eldest child had normal V A and the optic fundi were normal looking It was not possible to examine the eyes of the two youngest children but none showed any sign of reduced V A

B The Parents and Other Relatives
The parents were both examined at the age of 60 They are *not* related

The father who is clinically well had no signs of diabetes mellitus i e no glycosuria a normal fasting blood sugar value *and a normal glucose tolerance test He had* normal V A and the fundus of the eye showed a normal picture Blood group B C+ D— E+ c+ e+ K— MN ss Le^a— Le^b+ Fy^a—

He is the third of four sisters and brothers One of the brothers died as a child of unknown cause One sister and one brother have both beeen examined on our initiative and found to have *normal* glucose tolerance test normal V A and apparently optic fundi The paternal grandfather died at the age of 70 of pancreatic carcinoma The paternal grandmother died at the age of 7? None of them had diabetes or showed any eye disturbances

The mother who is clinically healthy had no glycosuria a normal fasting blood sugar and a normal glucose tolerance test Blood group B C+ D+ E— c—, K— MN Ss Le^a— Le^b+, Fy^a+

She is the third of four sisters and brothers Two of them died in childhood of unknown causes Her brother — now living in Canada — had according to his physician at the age of 66 a normal fasting blood sugar and no visual disturbances The maternal grandfather had at the age of 78 a thrombosis of the central vein in the left eye but no other eye disease He had no diabetes mellitus The maternal grandmother died aged 75 years and had neither diabetes mellitus nor any symptoms from the eyes

It must be noted that the three uncles and aunts all show involuntary sterility

Discussion

The pertinent observations in the present report may be summarized thus

In a family *without known heredity for diabetes* four siblings out of five (one boy and three girls) develop a *diabetes* at about the same age (15—18 years) and all the diabetic siblings prove in addition to have an *optic atrophy* of the primary type with defective colour vision The eldest sister, now aged 33 seems to be healthy in all respects and has three healthy children

Apart from the optic atrophy the siblings presented little of neurologic interest An undulating nystagmus was present only in the boy We should like to stress however the presence of a peculiar psychic instability in the affected siblings They are all intelligent and have performed well in school and university careers but have shown difficulties in establishing normal emotional contacts Thus two of the girls have for

periods refused medical control of their diabetes in a way most unusual in this country

The *diabetes*, which has now been present for 14 years in case 4 and since one year in case 1 is of the ordinary juvenile type without peculiar features It is still rather mild in case 1 who is doing rather well without insulin, but the sisters need insulin and two of them (cases 3 and 4) have a rather advanced diabetic retinopathy with hemorrhages and exudates to which we are prone to refer the central scotoma noted in case 4 This case has also developed a bilateral posterior cataract

The *optic atrophy* was present in case 1 one year before the onset of clinical diabetes it was searched for and detected in the other diabetic siblings only after the onset of diabetes in case 1 We can thus know nothing of the time when the optic atrophy had developed in these cases Some very discrete retinal changes noted in cases 1, 2 and 3 (macular granulation and slight pigment condensation in the periphery) may well be related to the optic atrophy

As far as we have been able to trace their ancestry the parents of the siblings are unrelated

The observations in the present family agree closely with those previously reported in cases with the constellation primary optic atrophy and diabetes mellitus The tendency to affect siblings is a striking feature of the syndrome and strong evidence for its being a specific genetic entity In a few of the families reported (3 11, 20) cases with diabetes mellitus alone were noted also in other relatives in no family however has optic atrophy alone been observed among the relatives

Bilateral nerve deafness was observed in 13 of the cases described in previous papers (though not present in our series), it seems justified to regard this lesion as a true third element of the syndrome (1 3, 11, 17, 20) Other neurologic signs have also been noted with a lower incidence It is of special interest that five patients in three families were noted to have the clinical picture of Friedreich s ataxia Psychic disturbances have been noted by several authors and were present in our cases The atony of the urinary bladder noted by Wolfram (20), and Shaw and Duncan (11) must be regarded as a sign of doubtful specificity especially in view of the high rate of bladder disturbances noted by Bartley et al (4) in a general material of diabetics

The *diabetes* has offered no peculiar features in cases hitherto published It appears as an ordinary juvenile diabetes which later may develop a retinopathy of the classic type In routine clinical work it may thus be easy to overlook the optic atrophy or to incorporate it into the general concept of diabetic retinopathy or neuropathy However it seems evident to us as to previous workers that the mechanism of the optic atrophy in these cases must be fundamentally different from that of the more common late diabetic complications mentioned above This opinion is supported by 1) the rare occurrence of optic atrophy in a general material of diabetics (19) 2) the specific type of familial occurrence of the syndrome 3) the high incidence of pre diabetic onset of the

optic atrophy, this being present before the onset of diabetes in 5/27 cases (1, 9, 18, 20) It is of interest to note also that the bilateral nerve deafness was present before the debut of the diabetes in 4/13 cases (1 11)

The primary optic atrophy may thus be regarded as the specific signal of the syndrome and deserves a special attention It seems in this condition to be a slowly progressing affection, thus resembling some other types of optic atrophy which are usually seen together with a retinal lesion of retinitis pigmentosa type and which are known to occur in various other hereditary syndromes (Laurence—Moon—Biedl s syndrome and others) A full blown picture of retinitis pigmentosa has not been observed in the present syndrome, but the rather discrete type of retinal lesion noted in our cases 1 2 and 3 and in some of the published cases seems akin to this condition It is definitely different from the group of hereditary optic atrophies usually cited as Leber s type

Diabetes may be present in some cases of Laurence—Moon—Biedl s syndrome we believe however, that the relations between the present syndrome and Friedreich s ataxia may be of greater interest

According to Thoren (14) a diabetes mellitus is found in approximately 20 % of all the cases with Friedreich s ataxia Also optic atrophy is sometimes seen in this disease In his large casuistic series of Friedreich s ataxia Thoren (15) found five cases with both diabetes and optic atrophy Two of them were siblings the others were mutually unrelated The optic nerve atrophy was present in 5/12 cases *with* diabetes and in 1/44 cases *without* diabetes within the Thoren series of Friedreich s ataxia

This disease is inherited in an autosomal recessive manner In cases with the combination diabetes optic atrophy Thoren has proposed, as a working hypothesis that the optic atrophy might be caused by the Friedreich gene' and the diabetes by another gene, perhaps linked in some way to the former (16)

In the published casuistics for the present syndrome (familiar diabetes + optic atrophy) we found it combined with Friedreich s ataxia in 3/11 families In a few of the 11 families there were some members with diabetes alone but in no case was optic atrophy alone observed It is thus possible that an hypothetical independent gene for diabetes may affect the penetrance of the gene governing optic atrophy (related to the Friedreich gene') Similarly the high incidence of diabetes in Friedreich's ataxia (20 %) makes it tempting to assume that the Friedreich gene also has a penetrance favouring effect making heterozygotes develop diabetes the diabetes gene was calculated to occur in 20 % of a general population (13)

A similar interplay of several genetic factors might be present also in other compound syndromes, in which a diabetes, clinically without peculiar features forms a more or less obligatory element To elucidate the controversial problem of heredity in diabetes mellitus a thorough knowledge of such compound syndromes may become of decisive importance

Summary

A family is reported in which four consecutive siblings out of five developed a diabetes mellitus at the ages 14—18, and also a primary optic atrophy, in one of the siblings one year before the onset of diabetes. The parents were not consanguineous, and no case of diabetes mellitus or optic atrophy was known either in the father's or in the mother's family.

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Leukaemia, Leukaemoid Reaction and Tuberculosis

By

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The blood alterations in active tuberculosis are usually mild ones such as moderate anaemia and leucopenia (25 33) Pronounced changes indicating leukaemoid reaction (21) or true leukaemia may occur however The clinical differential diagnosis in these cases is often difficult but is important for the treatment A retrospective study was therefore made on patients presenting diagnostic problems of this kind at Karolinska Sjukhuset and this group of patients was compared with similar patients reported in the literature with leukaemoid reactions reminiscent of myeloid (5 11 14 22 23) lymphatic (10 12 31) or monocytic leukaemia (13)

Material

The group of earlier cases from the literature numbered 25 (15 men ten women) ranging in age from 16 to 73 years they were reported between 1930 and 1954 (1 3 5 7 10 11 12 13 14 16 20 31 32) Confining the time period to these limits gives a series of patients which have not been treated with steroids and may be compared with the patients at Karolinska Sjukhuset almost all treated with steroids

The authors group comprised 11 cases (four men seven women) aged from 26 to 68 years they were admitted to Karolinska Sjukhuset and came to autopsy between 1952 and 1963

Definitions

The diagnoses have been based on examination of smears of peripheral blood and bone marrow taken during life and on histological sections of bone marrow and several other organs taken at autopsy The generally accepted criteria for the diagnosis leukaemia have been applied i e hypercellular bone marrow with the histological and cytological features of leukaemic disease abundant myeloid cells in various stages of maturity plus many small megakaryocytes in the cases of chronic myeloid leukaemia or dominance of atypical immature myeloid cells with obvious maturation arrest in acute myeloid leukaemia Cellular infiltrations in other organs at au

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topsy have supported the diagnoses but no diagnoses have been made on the basis of the findings in extramedullary tissues only In lymphatic leukaemia heavy diffuse infiltration of the bone marrow with mature or immature lymphoid cells has been the criterion supported by the presence of the cells in the peripheral blood and by nodular lymphatic infiltration in the liver at autopsy

The diagnosis leukaemoid reaction , on the other hand has been based on the absence of the above mentioned histological and cytological findings in the bone marrow in spite of a blood picture indicative of leukaemia In none of these cases however has the total number of white cells exceeded 30 000 mm³ The final diagnosis leukaemoid reaction has also been supported by the fact that those patients have not manifested an unequivocal neoplastic proliferation of cells leading to complete replacement of the bone marrow with leukaemic cells

Results

Old material

Leukaemia was found at autopsy in seven out of the 25 cases with a clinical diagnosis of leukaemia collected from the literature Data from marrow examination performed during life was available in only eight cases At autopsy there was evidence of active tuberculosis in all cases, but only in three was it known during life

Of the seven cases where there was leukaemia at autopsy one had known, healed tuberculosis one active tuberculosis and five clinically unknown tuberculosis Of 18 cases with a leukaemoid reaction at autopsy 14 had clinically unknown tuberculosis, two known healed tuberculosis and two active tuberculosis (table I) In almost all the cases the tuberculosis was disseminated located usually in the lungs, lymph nodes liver spleen and bone marrow The tubercular foci generally displayed a non reactive histologic picture, with necrosis but little if any adjacent cell reaction and with numerous acid fast bacilli In none of the cases had steroid therapy been given

The typical case in the old material thus had active tuberculosis, undiagnosed during life with a secondary leukaemoid reaction These patients had not received steroids

TABLE I Cases of tuberculosis and leukaemia collected from the literature between 1930 and 1954

	Tuberculosis		
	Cured	Active	Unknown
A Clinical diagnoses			
Leukaemia			
Myeloid	2	2	15
Lymphatic	1	1	3
Monocytoid			1

	Tuberculosis	
	Active pulmonary	Active disseminated
B Autopsy diagnoses		
Leukaemia		
Myeloid		6
Lymphatic		1
Leukaemoid reaction	1	17

A comparison is made between clinical and autopsy diagnoses All 25 cases were diagnosed as leukaemia during life but this diagnosis was confirmed at autopsy in only seven cases At autopsy all cases had an active tuberculosis but this was clinically unknown in 19 cases The typical case thus had an active tuberculosis undiagnosed during life and a secondary leukaemoid reaction

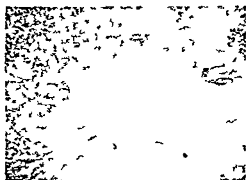
TABLE II Cases of tuberculosis and leukaem a treated at Karol nska Sjukhuset between 1952 and 1963

	Tuberculos s		
	Cured	Acti e	Un known
A Cl nical diagnoses			
Leukaem a			
Myelo d	6	1	2
Lymphat c	1	1	
	Tuberculosis		
	Act e pulmonary	Act e dissem nated	Cured
B Autopsy d agnoses			
Leukaem a			
Myelo d	1	3	3
Lympnat c			1
Leukaemo d react on	1		2

A comparison s made between cl nical and autopsy d agnoses All 11 cases were d agnosed as leukaem a during l fe and this d agnosis was confirmed at autopsy in e ght cases At autopsy fi e cases had an act e tuberculos s of wh ch only two were cl n cally und agnosed The typ cal case thus had a true leukaemia and most of en an inact e tuberculos s

Present material

In eight of the 11 cases comprising the authors group with a cl nical diagnosis of leukaemia this disease was confirmed at autopsy In all 11 cases bone marrow examination had been performed during l fe In the remain ng three cases the clinical d agnosis of leukaemia was un certa n



F g 1 Sect on of l er from a case diagnosed cl n cally a myeloblast c leukaemia No h story or susp c on of tuberculo s Stero d hormones ha e been g en Autopsy revealed myeloblast c leukaem a with sparse leukaem c inf ltration of the organs and d ssem nated non react e caseous tuberculo s w th large amounts of ac d fast bacill In the lower half of the sec t on there s a focus of caseous necros s Only occas onal lymphocytes can be seen n the v c n ty of th s focus and there are no ep thelo d or g ant cell There is severe fatty inf ltrat on H E × 25



F g 2 Sect on of spleen of the same case as in f g 1 Confluent caseous necrot c areas are seen in the lower part of the photograph but there is no ev dence of a spec f c cellular react on Z ehl Neelsen sta n revealed num r ous ac d fast rods in the arious o gans H E × 25

At autopsy five cases displayed active tuberculosis and six evidence of cured tuberculosis Of the four cases with leukaemia and active tuberc losis at autopsy t o had known healed pul

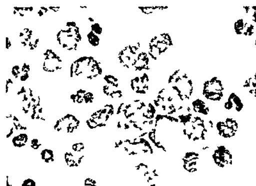


Fig 3 Smear of bone marrow from a case with the clinical diagnosis of PAS and INH treated tuberculosis of cervical lymph nodes and leukaemoid bone marrow reaction or sub-acute myeloid leukaemia Autopsy disclosed no evidence of active tuberculosis There was no leukaemic infiltration of the organs The bone marrow was hyperplastic The field shows that irregular myeloid cells with slight nuclear atypia predominate Toxic granulation is evident All stages of maturation up to granulocytes are present and there is no hiatus leukaemicus May Grunewald Giemsa × 250

TABLE III Comparison between the two materials with respect to diagnosis of tuberculosis and leukaemia during life and at autopsy

	No of cases of active tuberculosis		No of cases of leukaemia	
Material	During life	Post mortem	Post mortem	During life
1930—1954	3	25	7	25
1952—1963	2	5	8	11

This table shows that the clinical diagnosis of leukaemia was confirmed at autopsy in only seven out of 25 cases in the older material as compared with eight out of 11 cases in the present material In only three out of 25 cases was there a correct diagnosis of active tuberculosis during life in the older material as compared with two out of five cases in the later group

monary tuberculosis, but the active nature had not been suspected during life and the other two had clinically unknown tuberculosis (table II and figs 1 and 2)

The three cases with clinically uncertain diagnoses of leukaemia displayed a leukaemoid reaction with bone marrow suppression At autopsy no leukaemic infiltrate was found All three had a clinically evident tuberculosis, and in two the disease was strongly suspected to be active, but there was no evidence from cultures during life All three cases had received treatment for tuberculosis The autopsies of the respective cases revealed active pulmonary tuberculosis sepsis and lymph node tuberculosis (fig 3)

All but one of the 11 patients had received steroid hormones for the blood disease

The typical cases in the present material thus had steroid treated leukaemia and most often inactive tuberculosis

A comparison of the two materials with respect to diagnosis of tuberculosis and leukaemia during life and at autopsy is given in table III

Discussion

The difference between the two groups is probably due in part to the differences in treatment and diagnosis None of the earlier series had received steroid therapy, against all but one of the more recent group In only one third of the

earlier cases had a sternal puncture been performed but in all of the later group moreover modern tuberculostatics will naturally have been used much less often in the older material

The mechanism of the usually myelocytic leukaemoid reaction is as little understood as that of the normal leukocytosis in infection It is interesting to speculate on the association between maturation defects in the bone marrow and the effect of chronic inflammatory conditions on folic acid metabolism A reduced folic acid concentration is often seen in patients with chronic inflammatory conditions (6)

It is also interesting to note that tuberculosis has been reported in association with other haematological disorders such as myelofibrosis (4) polycythaemia (9) leucopenia (2) and pancytopenia (24)

In an examination of a possible causal association between blood disorders and tuberculosis it is of interest to establish which disease appears first In the earlier material this was not always indicated In the present group seven of the 11 had a history of cured tuberculosis but no activity was established by cultures at the time the blood changes were discovered

To summarize perhaps tendentiously the combination of tuberculosis and a leukaemic blood picture was previously said to entail a clinically unknown disseminated histologically non reactive tuberculosis with a blood picture that was identified on clinical grounds as leukaemia though this diagnosis was not confirmed at autopsy The same combination is ascribed in the present series to a cured tuberculosis that becomes activated during the treatment of leukaemia with steroids The tuberculosis in the present material was often hardly active Leukaemia was the prime haematological feature of the present series in only three out of the 11 cases was there uncertainty in this respect The present findings underline the need to check for tuberculosis when steroid therapy is being introduced in the case of leukaemia The possibility of routine use of tuberculostatics deserves consideration

Summary

A study has been made of patients with tuberculosis and blood changes resembling those in leukaemia Comparison of a group comprising 25 cases from the literature between 1930 and 1954 with a more recent group of 11 cases at Karolinska Sjukhuset from 1952 to 1963 reveals certain differences between the two groups

Most of the earlier cases had activ tuberculosis although they had receiv d no steroid therapy and there was a l u kaemoid reaction *In the pres nt g* the patients with a h story of cur l t berculosis who had received st treatment for leukaemia [illegible] rence of tuberculosis It th r f to be important to con d [illegible] treatment for tuberculos [illegible] able when introduc n st [illegible] in cases of leukaemia

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From Medical Department VIII (Head A Jervell, M D), Ullevaal Hospital Oslo, Norway

Coronary Artery Stenosis Murmur

A case report

By

PER G LUND LARSEN

In available textbooks on heart sound (2, 3, 5, 6, 8 11) and in papers on heart murmurs (1, 4, 7, 9, 10, 12) the possibility of murmurs from stenosed coronary arteries seems to have been neither mentioned nor discussed

Over stenosed peripheral arteries of the same diameter as the main coronary arteries, the occurrence of systolic and systolic diastolic murmurs is well known The flow pattern, pressures and environments are different in coronary and peripheral arteries, but these differences do not exclude the possibility that a coronary stenosis can generate murmurs In the left coronary artery such a murmur must be diastolic because of the predominant diastolic flow In the right coronary artery the conditions are somewhat different (2, 7, 11) and a systolic, systolic diastolic or a continuous murmur is a possibility

A case is now reported in which a high pitched diastolic murmur was obviously caused by a stenosis in the left coronary artery

Submitted for publication March 17 1967

Case report

A 75 year old woman was admitted to the Hospital because of a left sided cerebral thrombosis Many members of her family including two daughters and a son had either died from or had coronary artery disease

The patient had suffered from angina pectoris since she was 60 At 62 she had a posterior myocardial infarction

Physical examination

On admission she had a right sided hemiplegia Her B P was 140/90 mm Hg By auscultation a diastolic murmur was heard in a restricted area with the punctum maximum in the fourth intercostal space at the left sternal border The phonocardiogram (fig 1) showed that the murmur began about 0 06 sec after the second heart sound and ended before the first sound It was of the short crescendo long decrescendo type and in accordance with its high pitched musical character it was only seen in the 200 and 400 cycle/sec band The murmur became stronger after light physical exercise The ECG (fig 2) indicated an old posterior infarction From the X rays the heart volume was calculated to be 490 ml/m²

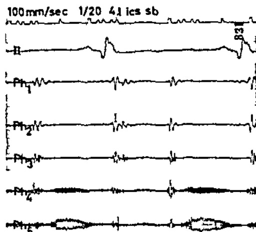


Fig 1 Phonocardiogram registrated by an Elema Schonander phonocardiograph with EMT 22 filter and EMT 25 microphone The frequencies corresponding to the filters Ph1 to Ph5 are 25 50 100 200 and 400 cycles per sec



Fig 3 Selective coronary angiography of the left coronary artery

body surface Fluoroscopy revealed some calcifications in the heart, and on cine fluoroscopy these densities were seen to be located in the left coronary artery Selective coronary angiography (fig 3) showed stenoses of both the left coronary artery trunk

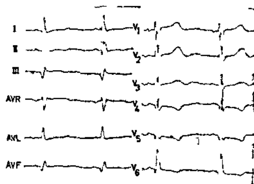


Fig 2 ECG 7 7 66 before the murmur disappeared

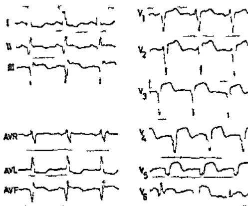


Fig 4 ECG 31 8 66 after the murmur had disappeared

and the arteria descendens anterior without any post stenotic dilation There was no shunt from the left coronary artery to the left ventricle and no coronary artery aneurism (The angiography was performed by dr Hoel Ulleval Hospital X ray Dept)

Sixteen days after the angiography was performed the patient got severe chest pain An ECG (fig 4) now revealed an acute anteroseptal infarction From this incident on the diastolic murmur could no longer be heard The BP, however was unchanged from before the infarction Six days later the patient suddenly died

Autopsy

A hemopericardium with heart tamponade explained the sudden death The heart weighed 430 g The left ventricle was slightly dilated The ostiae and valves were all normal An old fibrotic posterior infarction was found The left coronary artery and ramus descendens anterior were severely atherosclerotic with stenoses Corresponding to the stenosis in the arteria descendens anterior a fresh thrombus occluded the lumen In the interventricular septum and the anterior wall of the left ventricle there was a fresh infarction with a rupture to the pericardium

Discussion

The diastolic murmur heard in this patient must be explained as a coronary artery stenosis murmur This interpretation is confirmed by the coronary angiography and the quite normal heart valves and ostiae found at autopsy The final proof was the disappearance of the murmur in connection with an acute coronary thrombosis on the site of the stenosis

The diastolic murmur was characterised by its high pitch its localisation to a very restricted area, and the diamond shape and high frequency in the phonocardiogram

Summary and conclusion

In a 75 year old woman suffering from angina pectoris a high pitched diastolic murmur was heard in a restricted precordial area Selective coronary angiography showed stenoses of the left coronary artery The stenosed artery was reckoned to be the origin of the murmur as was confirmed by the disappearance of the murmur in connection with an acute myocardial infarction and by the autopsy finding of a coronary stenosis with acute thrombotic occlusion but otherwise quite normal valves and ostiae

The possibility of a coronary artery stenosis murmur should therefore be kept in mind when the causes of high pitched diastolic murmurs are discussed

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The Effect of Erythritol-tetra-nicotinate on Serum-cholesterol Levels in Man

By

D Brox and O Selvaag

Altschul et al in 1955 (2) showed that in man nicotinic acid in high dosage (3 to 6 g daily) could lower the serum cholesterol level significantly The occurrence of side-effects with such large doses of nicotinic acid has, however, considerably restricted its therapeutic use Attempts have therefore been made to produce a derivative of nicotinic acid with equal efficacy in lowering blood cholesterol but with less side-action Erythritol tetra nicotinate was synthetized by AB Bofors, Bofors, and named 8 AL This report deals with a clinical trial of this compound in 24 patients In 17 of these patients termed *Group 1* below, the main intention was to study the effect on the serum cholesterol level and the side-effects The other seven patients termed *Group 2*, are part of a series of patients treated for atherosclerotic disease and followed with respect to serum cholesterol All 24 patients had a level more than 300 mg%

Submitted for publication March 21 1967

Group 1

Methods

The trial started with a period of a minimum of six weeks in which the patients were put on a low fat diet to which was added one spoonful of cod liver oil or soy bean oil daily This was called *the first control period* After this the patients were put on 3 g of 8 AL daily during two periods each of six weeks duration. 8 AL was given in tablets containing 0 5 g and preferably in a dose of 1 g three times daily after meals Between the two periods of treatment there was a control period of six weeks—*the second control period*

Before the trial and at the end of both treatment periods the following laboratory estimations were carried out urinary sugar SGOT Meulengracht thymol turbidity test alkaline phosphatase trombotest (TT) white blood cells including a differential and thrombocytes At the beginning of the trial the patients were examined clinically their basal metabolic rate determined and an ECG (12 leads) recorded During the trial the serum cholesterol levels were

checked fortnightly, by the method of Carr and Drekter (6) Blood samples were taken between breakfast and lunch

Material

Most of the 17 patients were suffering from obliterative atherosclerosis and/or coronary heart disease Cases with diabetes or myxedema were excluded There were eight men with a mean age of 53 years (40 to 62 years) and nine women with a mean age of 60 years (47 to 67 years)

Only seven patients four men (mean age 52 years) and three women (mean age 66 years) completed the trial according to plan One man and two women completed it on a reduced dosage of the drug Two men and one woman did not take part in the second treatment period, one man because he disliked drugs in any form the other two owing to side effects of the drug The remaining four patients discontinued treatment early one owing to a fractured leg which prevented regular checks and three women owing to side effects

The women who did *not complete the* trial according to plan were on an average nine years younger (57 years)

Results

Side effects

Most of the patients experienced flushing of more or less severity during the first few days or weeks, especially when the drug was taken between meals

The more severe complaints leading to reduction of dosage or to stopping of the trial were as follows

Flush alone	1 woman
Flush + severe itching	2 women
Flush + 'white fingers	1 woman
Flush + dyspepsia	2 women
Feeling of coldness itching and bad taste in the mouth	1 man

Those who responded well have continued the treatment One man aged 40 years developed, however, a pyloric ulcer after nine to ten months' therapy

Effects on serum cholesterol levels

Table I shows the cholesterol values in the control and treatment periods for those who completed the whole trial

With occasional exceptions the values are the mean of three samples taken two weeks apart During the first period of treatment the mean cholesterol value was reduced by 101 mg% (a reduction of 27,8 %) During the second control period there was an increase of 51 mg% and during the second treatment period a further fall of 41 mg% The difference between the two treatment periods seems negligible

During the second control period the mean cholesterol value did not rise to pre treatment level This may be due to a prolonged effect of the drug Table II shows that the increase took place within two weeks after stoppage of the drug

Fluctuations, possibly spontaneous, occurred during both treatment and control periods in several patients

The results in patients on a reduced dose of the drug are shown in table III

During the second control period there was again a variable increase in cholesterol

Three of the patients completed only the first treatment period with or without reduction of dosage (table IV)

The remaining four patients took the drug for too short a period to allow of inclusion in the analysis of results

Liver function bone marrow (white blood cells, thrombocytes) and urine

TABLE I Mean serum-cholesterol levels (mg%) during the different observation periods in the seven patients who completed the trial according to plan

Pat no	1st control period	1st treatment period	2nd control period	2nd treatment period
1	389	292	311	282
2	345	245	295	234
4	357	249	279	261
5	329	264	284	295
6	435	307	361	373
7	315	211	279	218
15	373	268	385	244
Mean ± SEM	363 ± 15	262 ± 12	313 ± 16	272 ± 20

t tests on individual differences

1st control period	vs 1st treatment period	$p < 0.001$
1st treatment period	vs 2nd control period	$p < 0.01$
2nd control period	vs 2nd treatment period	$p < 0.05$
1st control period	vs 2nd treatment period	$p < 0.001$
1st control period	vs 2nd control period	$p < 0.01$
1st treatment period	vs 2nd treatment period	N S

TABLE II Cholesterol values (mg%) during the second control period

Pat no	Start[1]	After 2 weeks	After 4 weeks	After 6 weeks
1	292	310	312	310
2	245	292	305	288
4	249	270	265	302
5	264	302	288	261
6	307	335	370	378
7	211	223	305	310
15	268	380	415	360
Mean ± SEM	262 ± 12	302 ± 19	323 ± 19	316 ± 15

[1] Start = 1st treatment period in table I

seemed normal during treatment, except in one patient in whom the SGOT increased from 20 to 55 units followed by a decrease to 23 units when treatment was discontinued

The lowering of cholesterol by 27.8% in those patients who completed the trial agrees closely with published results obtained with nicotinic acid in a dose of 3 g daily (table V)

TABLE III Mean serum cholesterol values (mg %) in three patients on reduced doses of 8 AL

Pat no	Dose of 8 AL daily (g)	1st control period	1st treatment period	2nd control period	2nd treatment period
3	2	334	307	310	295
8	1—2	545	508	546	493
9	1 5—2 5	456	326	316	325
Mean		445	380	391	371

TABLE IV Mean serum-cholesterol values (mg %) in three patients who completed the 1st treatment period only

Pat no	Dose of 8 AL daily (g)	1st control period	1st treatment period	2nd control period
10	3	330	216	255
11	1 5—3	317	265	315
12	1 5	394	355	366
Mean		347	279	312

TABLE V Percentage reduction in serum cholesterol levels obtained in different series with nicotinic acid (3 g daily)

Authors	No of pat	Duration of treatment	Reduction obtained (%)	Remarks
Achor & al (1)	18	3 months	12	Fam hyperchol
Altschul & Hoffer (3)	12	2 weeks	21 46	Healthy young adults
Berge & al (4)	51	3 months	19	
Chazin (7)	15	3 months	29 5	Cholesterol level > 250 mg %
Davis & al (8)	20	4 weeks	17	
Goldner & Vallan (9)	16	10 weeks	39	
Hunter	25	1 month	20	
O Reilly & al (12 13)	27	6 weeks	20 5	Without arteriosclerosis
	10	6 weeks	21 1	With arteriosclerosis
Parsons (14)	26	30 weeks	20 3	Mean cholesterol level 325 mg %
	16	30 weeks	16 6	Mean cholesterol level 312 mg %
Öst[1] (16)	22	2—8 months	21	

[1] 3—5 g

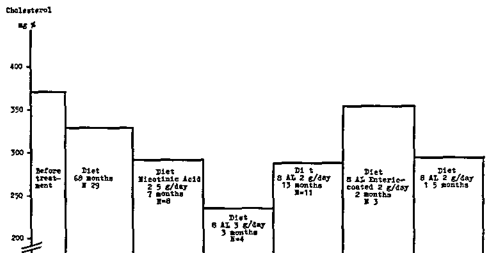


Fig 1 Patient no 2 The effect of a cholesterol reducing diet with and without add tion of pure nicotinic acid or 8 AL in different doses 8 AL administered uncoated or enteric-coated N number of samples

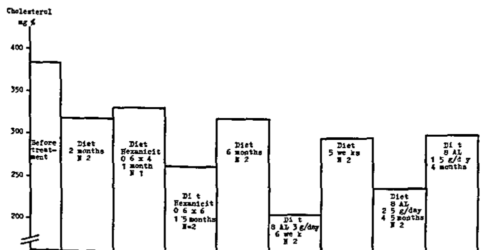


Fig 2 Pat ent no 4 The effect of a cholesterol reducing d et with and without addition of inositol nicotinate (Hexanicit) or 8 AL in different doses N number of samples

Group 2

In seven patients from another series of cases with intermittent claudication a similar lowering effect on the serum cholesterol has been recorded In four of these patients, not previously on a diet treatment for on average 7 5 months lowered the mean cholesterol value from 413 to 282 mg% a reduction of 31 % In two patients the mean cholesterol value during a period of cholesterol lowering diet was reduced from 354 to 323 mg% Following the addition of

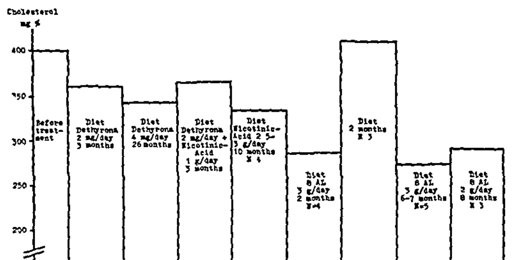


Fig 3 Patient no 1 The effect of moderately effective cholesterol reducing diet with and without addition of dextrothyroxinesodium (Dethyrona®) pure nicotinic acid or 8 AL in different doses N — number of samples

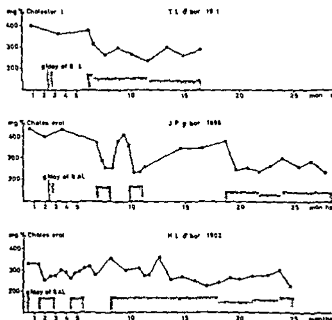


Fig 4 Serum cholesterol levels in three patients followed for more than one year

Hexanicit® (meso-inositol hexanicotinate) 3 g daily it was further reduced to 299 mg%, and following the substitution of Hexanicit for 8 AL it came down to 267 mg%, i e 56 mg% or 17 % below the diet period level The seventh patient previously on a cholesterol reducing diet, had a decrease in serum cholesterol from 382 to 278 mg% on a dose of 2 g 8 AL daily for five months

The effects of different therapies during more *prolonged observation periods* are demonstrated in figs 1, 2, 3 and 4 These also show how the degree of effect varies with the size of the dose of 8 AL Enteric coated tablets of 8 AL seem to be ineffective or less effective (fig 1)

Discussion

Although this material is small the trial indicates that the drug 8 AL is effective in lowering the serum cholesterol level in patients with a previous level more than 300 mg% Perhaps higher dosage might have demonstrated this still more clearly In equal doses it seems to be as effective in this respect as pure nicotinic acid

Side effects were, however, common Flushing and discomfort were more marked in middle aged women This may be fortuitous, the number of patients being small Perhaps a more gradual increase of dose would have reduced the incidence of these side effects as found by Carlson and Oro (5)

Given that drugs exist which lower serum cholesterol without serious side-effects and can be taken for years, the following questions arise what will be the clinical importance of these drugs and to whom should they eventually be given?

The long term prognosis in patients with myocardial infarction obviously hinges on a diet that can lower serum cholesterol (11) Nicotinic acid when given for years can not only increase the peripheral circulation, but also reduce atheromatosis in obliterative atherosclerosis Also angine d effort has been improved (16 18)

Reduction of serum cholesterol level in hypercholesterolemic patients is evidently advantageous It therefore seems reasonable to treat persons with hereditary hypercholesterolemia and xanthomatosis Further it seems worth while to try the treatment in patients with atherosclerotic diseases and high serum cholesterol, when a dietetic regimen has failed to reduce the cholesterol level satisfactorily

Summary

A new derivative of nicotinic acid (erythritol tetra nicotinate) called 8 AL (Bofors) has been shown, in a trial with 24 patients, to reduce the serum cholesterol level as effectively as pure nicotinic acid Unfortunately side-effects are not uncommon with this compound too Enteric coated tablets of 8 AL were found ineffective

The role of a regimen or drugs that reduce serum cholesterol is briefly discussed

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Chromosome Studies in Patients Treated With Azathioprine and Amethopterin

By

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The occurrence of chromosome abnormalities in acute leukaemia has been well established At the same time it has become apparent that various cytotoxic drugs produce structural changes of chromosomes in human cells both in vivo and in vitro Consequently, the chromosomal aberrations in patients with acute leukaemia treated with cytotoxics must be interpreted with caution are they a result of the leukaemic process or the cytotoxic treatment? The present report describes the effects of two cytotoxic drugs, azathioprine (Imuran®) and amethopterin (Methotrexate®), on chromosome morphology in man

Azathioprine and amethopterin were studied for the following reasons

1) 6 mercaptopurine and amethopterin are the most widely used cytotoxic drugs in the treatment of acute leukaemia Azathioprine is a S substituted derivative of 6 mercaptopurine It is believed that thioglucosidases in the organism split the S linkage of azathioprine and the cytotoxic and immunosuppressive effect of the drug is thus at least partly due to the presence of 6 mercaptopurine in the tissues Consequently investigation with azathioprine may clarify the role of 6 mercaptopurine in producing the chromosome aberrations which have been demonstrated in leukaemic patients treated with this drug

2) During the last few years cytotoxics have been used to an increasing extent in the treatment of the autoimmune diseases and psoriasis Azathioprine and folic acid antagonists are the most frequently employed drugs for this purpose It is important, therefore to clarify whether they will produce chromosomal changes in human cells

Material and methods

Six patients treated with azathioprine and four patients treated with amethopterin were studied The *azathioprine treated* patients were all females aged between 29 and 59 years Four of them suffered from cirrhosis of the liver The remaining two

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patients had disseminated lupus erythema tosus and Wegener's granulomatosis respectively. None of the patients had previously been treated with cytotoxics. The patients were treated with prednisone in doses of 5 to 50 mg daily at the beginning of the present study and had received this treatment for seven days to 21 1/2 months.

Chromosome studies were performed on bone marrow aspirates from each patient *before* therapy with azathioprine was instituted. Bone marrow aspirates were repeated in all patients at least once during the cytotoxic treatment and also in two patients after cessation of azathioprine therapy. Lymphocytes from the peripheral blood were cultured when the marrow aspirates were obtained. Unfortunately some cultures failed to grow.

The four *amethopterin treated* patients all had psoriasis. Two were male and two female and their ages ranged from 17 to 69 years. Two of the patients (nos. 1 and 3) had received 25 mg of amethopterin 11 1/2 months and 1 1/2 and 3 weeks prior to the present study respectively. Bone marrow aspirates were obtained before and one to five days after the administration of 25—50 mg of amethopterin intramuscularly.

As controls the marrow aspirates of five patients without cancer or any haematologic disorder were cytogenetically investigated. Chromosome studies were also performed on the peripheral blood cells of five healthy volunteers.

The marrow aspirates were treated according to a modification of the technique described by Tjio and Whang (35). The cells of the peripheral blood were cultured according to a slight modification of the method of Moorhead et al. (19). The cultures were harvested after 72 hours.

Results

1. Azathioprine Study

A. *Bone marrow cells*

Table I shows the chromosomal findings in the marrow aspirates and the amount of azathioprine given. The marrow aspirates had diploid modes, but some hypodiploid cells were met in all preparations. The hypodiploidy was due to chromosomes missing from different groups. When the results depicted in table I are compared with the results of chromosome studies performed on bone marrow aspirates and peripheral blood cells from the controls (table II) it is seen that neither prednisone nor azathioprine therapy produced increased aneuploidy. However, in patient no. 2 the marrow aspirate obtained during azathioprine therapy contained eight polyploid cells per 100 metaphases scored.

Before treatment with azathioprine was instituted 0—4 % of the metaphases contained structural chromosome abnormalities. These figures do not exceed those found in the controls. In contrast, an increased number of metaphases with structural chromosome aberrations were found in marrows obtained during azathioprine therapy. The difference between the total number of metaphases with chromosome aberrations in the marrow aspirates obtained before and during therapy with azathioprine is highly significant ($chi^2 = 16.73$, $p < 0.001$).

In two of the patients bone marrow aspirates were obtained 14 days and 7 and 9 1/2 months, respectively after cessation of azathioprine. At these times, the number of abnormal metaphases did not significantly exceed the pre-treatment values.

The chromosome aberrations consisted of breaks of the chromatid and chromosome types and acentric frag

TABLE I Chromosomal findings in bone marrow aspirates from patients treated with azathioprine

Pat no	Date	Relation to therapy with azathioprine	Amount of azathioprine given (mg)	Total cells scored	Chromosome number								Metaphases with structural abnormalities (%)
					<42	42	43	44	45	46	47	>47	
1	30 11 64	B	0	50	1	1	1	3	2	42			2
	21 12 64	D	3 150	50	3		1	2	4	40			8
	8 10 65	A	—	50				1	1	48			4
	9 11 65	D	2 850	50					3	47			12
	18 2 66	D	18 000	50					3	47			4
2	14 4 65	B	0	50					2	48			2
	30 4 65	D	2 100	50					2	48			24
	30 11 65	A	—	50				1	3	46			4
	17 2 66	A	—	50			1	2	2	44		1	4
3	12 7 65	B	0	50				1	4	45			4
	?6 7 65	D	2,100	50				1	1	48			6
4	7 8 65	B	0	50		1	1	1	1	45			0
	19 8 65	D	1 950	50					4	46			4
	3 12 65	D	12 400	50		1	2		4	41	2		12
5	20 12 65	B	0	50			1		7	42			2
	2 4 66	D	1 050	50					7	42	1		6
6	18 3 66	B	0	50	1				3	46			0
	15 4 66	D	2 250	50	1			1	2	46			8

A = after therapy
B = before therapy
D = during therapy

ments In the second bone marrow aspirate from patient no 2, metaphases with chromatid interchanges were seen (fig 1) In patient no 2 several metaphases showed structural changes of two or more chromosomes

B Cells cultured from blood

Successful cultures both prior to and during therapy with azathioprine, were obtained only in patients nos 5 and 6 The number of aneuploid metaphases or metaphases with structural aberrations did not increase significantly during cytotoxic therapy From patient no 2 lymphocytes from the peripheral blood were cultured 7 and 9 1/2 months after cessation of azathioprine In the first culture 8 out of 100 metaphases scored contained 47 chromosomes Three of these could be karyotyped The hyperdiploidy was due to a supernumerary chromosome with the size of one of the chromosomes of group 6 X 12 In the culture set up 2 1/2 months later hyperdiploid metaphases were no longer en

TABLE II Chromosomal findings in bone marrow aspirates and cultured lymphocytes of the peripheral blood from the controls

Pat no	Date	Type of tissue	Total cells counted	Chromosome number								Metaphases with structural abnormalities (%)
				<42	42	43	44	45	46	47	>47	
1	20 8 66	BM	50					3	47			2
2	28 9 66	BM	50					5	45			2
3	29 9 66	BM	50			1	1	5	43			0
4	14 10 66	BM	50				1	6	43			0
5	17 10 66	BM	50			1	2	3	43	1		0
6	2 4 66	PB	50			1	1		47	1		2
7	18 4 66	PB	50	1			2	3	43	1		4
8	25 5 66	PB	50		1			2	47			0
9	2 7 66	PB	50	1	1			5	42		1	2
10	5 8 66	PB	50	1				1	48			2

BM = bone marrow
PB peripheral blood

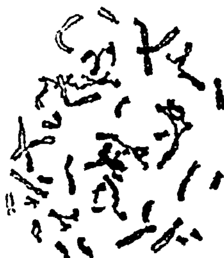


Fig 1 A cell from case 2 of the azathioprine study showing multiple breakage and chromatid interchanges.

countered Ten % of the metaphases of this culture contained structural chromosome aberrations The results of the chromosome studies in cultured lymphocytes are summarized in table III

2 AMETHOPTERIN STUDY

In this group, only bone marrow material was studied Table IV presents the chromosomal findings in the marrow before and during therapy with amethopterin The marrow cells had diploid modes In the hypodiploid cells chromosomes from different groups were missing The cytotoxic treatment produced no increased aneuploidy It is seen from table IV that from one to five days after the injection of amethopterin an

TABLE III Chromosomal findings in cultured lymphocytes of the peripheral blood from patients treated with azathioprine

Pat no	Date	Relation to therapy with azathioprine	Total cells scored	Chromosome number <42	42	43	44	45	46	47	>47	Metaphases with structural abnormalities (%)
2	30 11 65	A	100	1	1	1		11	78	8		4
	18 2 66	A	50			1	1	2	46			10
3	26 7 65	D	50				3	4	43			6
4	8 3 66	D	50			2		6	42			6
5	25 3 66	B	50	1			3	5	39		2	2
	2 4 66	D	50			1		4	45			2
6	18 3 66	B	50	1				2	47			2
	15 4 66	D	50					4	46			4

A = after therapy
B = before therapy
D = during therapy

TABLE IV Chromosomal findings in bone marrow aspirates from patients treated with amethopterin

Pat no	Date	Relation to therapy with amethopterin	Total cells scored	Chromosome number <42	42	43	44	45	46	47	>47	Metaphases with structural abnormalities (%)
1	17 2 66	B	50			1	1	3	45			2
	5 3 66	A	50			1	2	1	46			22
2	8 8 66	A	50			1		3	46			2
3	6 9 66	B[1]	50		1		1	2	46			0
	8 9 66	A	50			2		4	44			6
4	6 9 66	B	50			1	1	1	47			0
	12 9 66	A	50		1	1	2	2	44			10

[1] 25 mg amethopterin had been given i m 4 3 1965
[2] 25 mg amethopterin had been given i m one two and three weeks before

A = after therapy
B = before therapy



Fig 2 A cell from case 1 of the amethopterin study showing multiple small fragments and centromere spreading (arrows)

increased number of metaphases with structural chromosome abnormalities was present The difference between the total number of metaphases with structural chromosome aberrations in the marrow aspirates obtained before and after therapy with amethopterin is significant (chi² 11 63 p<0 001)

The chromosomal abnormalities observed were breaks of the chromatid and chromosome types and acentric fragments In patients nos 1 and 4 several metaphases contained abnormalities of two or more chromosomes One metaphase showing centromere spreading was seen (fig 2)

Discussion

A wide spectrum of abnormal chromosome patterns are found in acute leukaemia Structural aberrations and an increased number of pseudodiploid aneuploid, and polyploid metaphases are common (28) Abnormal stemlines of cells have been demonstrated in many cases (28) Finally, endoreduplication may be seen (14) These various types of chromosome abnormalities are met in untreated patients as well as in patients receiving treatment with cytotoxics

The present study provides information on the cytogenetic effect of three agents commonly employed in the treatment of acute leukaemia

1) Prednisone

The results of chromosome studies performed on bone marrow aspirates obtained from patients treated with prednisone alone indicate that glucocorticoids have no effect on chromosome number or morphology

2) 6 mercaptopurine

Few reports have dealt with the effect of 6-mercaptopurine on chromosomes and no studies have been performed on patients suffering from non malignant diseases treated with 6-mercaptopurine

In 1958 Biesele (2) demonstrated that 6 mercaptopurine had a chromosome breaking effect in mouse tissue cultures Pedersen (24) found breakage and an increased number of aneuploid and tetraploid metaphases in a skin biopsy from a patient with myeloblastic leukaemia treated with 6 mercaptopurine

In the light of these investigations and the present study of azathioprine the results of cytogenetic studies in acute leukaemia must be interpreted with caution when therapy with 6 mercaptopurine is given as both the leukaemic process and the cytotoxic treatment may

be respons ble for the structural ab normal t es and increased number of aneuplo d or polyplo d metapha es er countered In contrast abnormal cell l nes ha e not been demonstrated after treatment with 6 mercaptopurine or azath opr ne

The re ults of the present study indi cate that the amount of azath oprine g en does not correlate with the number of chromosome abnormal t es demon strated

3) Fol c acid antagon sts

In 1963 Taylor (33) demonstrated a chromosome break ng effect of am nopterin in cells of Vicia faba Recent ly Ryan et al (27) stud ed the chromo somal constitut on of cultured lymphocytes from the per pheral blood of 16 patients w th psor as s e ght of wl om had been treated w th fol c acid an tagon sts In tl e patients who had re ceived cytotox c therapy an increased number of metaphases w th cl romosome breakage abnormal chromosomes and hyperd plo d modes were demonstrated On the other hand Hampel et al (12) fa led to demonstrate any chromosome damag ng effect of amethopterin on human lymphocytes in v tro The present study conf rms the find ngs of Ryan et al (27) that amethopterin can produce structural chromosome aberrat ons n v o An increased number of ane plo d cells was not demonstrated Th s d crepancy of results ind cates that in vitro exper ments do not fully reflect drug effects on chromosomes in viv

In conclus on fol c ac d antag n sts n v o are able to prod e sever l chromo ome abnormal t es (str t ral abnormal ties and an ncrea e l t of aneuplo d and p e do l p lo l t phas s) w l cl are al o f n l n acut leukaem a In contrast al o l ll lines l ave not been fo n l f t e ment with tl ese dr gs

Possible Implications of Indu d Chromoso ie Aberrations

Bone marrow aplas a

It is generally agreed that w h ch damage chromo omes n a lead to cell deatl Several cl ro break ng agents sucl a ion n d t on benzene and cyt tox cs m duce marrow aplas a or de Damage to other tissu s s ch as th proliferating epitl el um of tl e d tract has also been described

In th s context t may be ment ne l that patient no 2 in the azatl prine study in wl om tl e cytotox c treatm en produced tl e most prono nced struc tural chromo ome cl anges was tl e nly pat ent in tl e ser es wl o developed a marked bone marrow depress on res lt ing in le kopen a (1 300 leukocytes/l) Tl e leukopenia d sappeared upon ces sation of therapy

Teratogene is

If damage to tl e genet c mater al f tl e cells leads t cell death or interference with cellular funct on one may a sume tl at cl r m som breaking agents m ht pos ess t rat gen c p opert es In fact such an effect has been dem nstra ed with ion n g rad at on certa n cyto t x cs and viruses A cl romosome dar ging effect of thal dom de er is

degradation products has been demonstrated in Allium cepa (10), Vicia faba (20), and human cells in vitro (16)

Neoplasia

The demonstration of abnormal chromosome patterns in human leukaemia and solid neoplasms has aroused new interest in the somatic mutation hypothesis originally proposed by Boveri (7), who suggested that abnormal chromosome complements might be the primary cause of cancer This hypothesis has recently been revised in some respects by several authors (13, 21)

Several recent observations suggest that changes in the chromosome constitution — visible or invisible — may predispose to or per se lead to the development of cancer

1 During the last few years it has been demonstrated that agents which are carcinogenic or leukaemogenic in man viz ionizing radiation and benzene, are able to produce structural chromosome aberrations in vivo in human cells (25 38 It has also been ascertained that several agents known to be carcinogenic in animals have a chromosome breaking effect in vivo or in vitro These agents are the nitrogen mustards (9) triethylene melamine 12 busulphan (3) aflatoxin (18), and certain viruses (22)

2 Abnormal chromosome patterns have been demonstrated in metaphases from carcinoma in situ lesions (30

3 Levan and Biesele (17) demonstrated that normal murine embryonic cells showed chromosomal instability on explantation in vitro Abnormal stem lines developed later Implantation of the cultured cells in mice produced spindle cell sarcomas

4 Recently it has been demonstrated that carcinogenic polycyclic hydrocarbons react with DNA in vivo to a greater extent than non carcinogenic hydrocarbons (8)

5 Leukaemia occurs significantly more often in conditions associated with chromosome abnormalities, for example in Down's syndrome (31) During the last few years it has been demonstrated that structural chromosome aberrations are present in the cultured lymphocytes from patients with constitutional aplastic anaemia (5) and Bloom's syndrome (29), both of these conditions appear to be associated with an increased incidence of leukaemia (4, 5) In a cytogenetic study of 25 children with leukaemia *in remission* Borges et al (6) found three patients with congenital aneuploidy Aneuploid cell lines have been demonstrated in some patients with aplastic anaemia polycythaemia, myeloid metaplasia, and atypical myeloid disorders (23) In several of these cases leukaemia developed later

6 It has been demonstrated that cells of the hamster line BHK 21 and the mouse line 3T3 are more susceptible to transformation by oncogenic viruses if, before infection, they are treated with ionizing radiation (32) and bromodeoxyuridine (36), respectively, both of which possess chromosome breaking ef

fects (15 38) Recently, Todaro et al (37) demonstrated that cell strains derived from skin biopsies from two patients with constitutional aplastic anaemia showed a much higher frequency of transformed colonies when infected with oncogenic viruses than cells from normal individuals

These observations suggest that abnormal chromosome complements are connected with the development of cancer Abnormal chromosome patterns — produced by chromosome breaking agents or arising spontaneously — might in some cases provide one or more cells with a selective proliferative advantage and autonomy compared with cells with normal chromosome complements Another possibility is that abnormal cell lines or cells with structural chromosome aberrations might be more susceptible to the action of oncogenic agents than cells with normal chromosome complements

Several objections have been raised to the somatic mutation hypothesis Normal chromosome patterns are often found in neoplasms of man and mammals However this finding needs not invalidate the hypothesis In several of the cases cited it cannot be excluded that the study was performed on chromosomes of normal cells present in the neoplasm It is also possible that chromosome abnormalities indetectable to light microscopy may be present in neoplasms with apparently normal chromosome patterns Another objection has been that a convincing connection between carcinogenicity and mutagenicity has not been demonstrated However the failure to establish a correlation between mutagenesis in monocellular organisms and carcinogenesis in mammals may be regarded as a reflection of the differences of the organisms

In the light of these possibilities it is well known that both the two agents dealt with in the present study viz azathioprine and amethopterin are able to produce aplasia of the bone marrow No information is available on a possible teratogenic effect of azathioprine in man but Githens et al (11) have recently demonstrated that the drug is able to produce malformations in mice Likewise 6-mercaptopurine is teratogenic in mammals (39) evidence of similar property in man has not yet been presented The teratogenic effect of folic acid antagonists has been demonstrated in man in connection with the employment of these agents as abortifacients (34)

It is difficult to evaluate a possible carcinogenic effect of chemical agents in man A prospective study of a large series of patients who have been treated with the drug in question is essential It is still more difficult to elucidate the problem when drugs, such as those used in the present series have mainly been used in the treatment of systemic malignancies with a grave prognosis Rees et al (26) recently reviewed a series of 171 patients with psoriasis in whom treatment with folic acid antagonists had been instituted at least 10 years ago and found one case of leukaemia This finding could, however, be fortuitous At the present time, data on a possible carcinogenic effect of folic acid antagonists in mammals are scarce However, Barich et al (1) demonstrated that aminopterin enhanced the carcinogenic

effect of 3 methylcholantrene on skin in mice

No information on possible carcinogenic effects of 6 mercaptopurine and azathioprine in man or mammals is available Azathioprine has been in therapeutic use in collagen diseases' for only a few years Compared with the latent interval of leukaemia arising from irradiation or benzene exposure, this period of observation is far too short to evaluate a possible carcinogenic effect of azathioprine in man

Although these theories are unproven it must nevertheless be emphasized that there is *considerable experimental* evidence which indicates that the administration of agents with a chromosome breaking effect in vivo in man may involve a potential carcinogenic and teratogenic risk The application of chromosome studies in mammalian tissues in vivo as a screening technique for possible *carcinogenic and teratogenic* effects deserves further consideration

Summary

Chromosome studies were performed on bone marrow aspirates from six patients with various auto-immune diseases treated with azathioprine and from four patients with psoriasis treated with amethopterin Both agents were shown to produce various types of chromosome breakage whereas no abnormal stemlines were seen

The possibility that the chromosome breaking ability of physical, chemical, and viral agents may be indicative of a possible teratogenic or carcinogenic effect is discussed

The present study was performed in order to investigate to what extent cytotoxic agents may participate in the production of the chromosome aberrations met in patients receiving cytotoxic therapy for acute leukaemia

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On the Demonstration of Bence Jones Proteinuria

By

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In recent years it has been established that Bence Jones protein (BJP) is a micromolecular paraprotein (14, 17) *The physico chemical properties of* BJP may vary considerably Differences in molecular weight, heat solubility salting out susceptibility and electro phoretic mobility may make it hard to demonstrate Bence Jones proteinuria (BJPU) (10)

Different modifications of the boiling test have previously been used to dem onstrate BJPU Precipitation in a weakly acidic solution at 45—60° C followed by re dissolution on boiling has been regarded as a criterion of BJPU Riva (14) was the first to demonstrate that thermosolubility does not always occur This detracts from the value of *the boiling test Paper electrophoresis* of urine is considered a better method for the demonstration of BJPU (5, 10 12 14 17)

The demonstration of BJPU is of great diagnostic significance in myelomatosis and macroglobulinaemia If BJPU is demonstrated, paraproteinaemia is probably present, even when the serum protein pattern is atypical, as is micro*gammamyeloma Because of its low* molecular weight, BJP is excreted by the kidneys If the clearance is high in re lation to the rate of formation, BJP will be low in serum and only demonstrable by immunological methods (11 13, 14) In such cases which account for ap proximately 20 % of all myelomas the demonstration of BJPU is of the greatest diagnostic significance

For some time we have used Phlippen s combined sulphosalicylic acid and boiling test The purpose of this paper is to draw attention to this method and to compare the results of the different methods in order to find the best one

Material and methods

The material consisted of 42 cases of mye lomatosis and one case of macroglobulinae mia Waldenstrom

The diagnosis was based on histological bone marrow examination paper electro

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6 *Paper electrophoresis of urine*

Paper electrophoresis was carried out at The Central Laboratory O ly City Hospital (present head O P Foss M D) Whenever the protein concentration assessed by Heller s reaction or the microbiuret reaction was low the urine was concentrated by ultrafiltration before the examination This procedure increased the protein concentration to the desired level of about 1 g/100 ml urine

A typical electrophoresis diagram in BJPU will show a dominating peak in the gamma region — the so-called M component — corresponding to the peak found on serum electrophoresis The mobility of the urine protein may be slightly faster than that of the corresponding serum constituent (10)

7 *Immune electrophoresis of urine*

Immune electrophoresis was carried out by M Harboe M D Institute for Experimental Medical Research Oslo City Hospital

Results

Table I compares the results of paper electrophoresis in serum and urine with the results of Jacobson and Milner s boiling test in 35 cases of myelomatosis BJPU was present in two cases where the boiling test was negative

Table II gives the incidence of positive results obtained with our routine methods as compared with paper electrophoresis in 43 cases of paraproteinaemia

HCl precipitation test was positive in 50 % without any false positive results

Salting out test was positive in only nine out of 22 cases Two urine specimens were cloudy and could not be tested

Jacobson and Milner s boiling test showed the greatest accuracy, apparently 100 % However we had two false positive and two false negative results in our myeloma cases, and also two false positives in urine from patients with other diseases

Osgood Haskins test gave one false

TABLE I Paper electrophoretic abnormality in serum and urine in relation to a positive boiling test in 35 cases of myeloma

Characteristic electrophoretic abnormality in	No of cases	No of cases with a positive boiling test
Serum only	13	0
Serum and urine	12	11
Urine only	10	9
Total	35	20

TABLE II Number of positive tests in urine with five different methods in 43 cases of paraproteinaemia

HCl precipitation test	11
Salting-out test	9
Jacobson and Miller s boiling test	22
Osgood Haskins test	7
Paper electrophoresis	22

TABLE III Number of positive tests employing nine different methods in 16 cases of BJPU

Heller s test	16
Sulphosalicylic acid precipitation test	16
Paper electrophoresis	16
Immune electrophoresis	16
Jacobson and Milner s boiling test	14
Philippen s boiling test	12
HCl precipitation test	8
Salting out test	5
Osgood Haskins test	4

TABLE IV Incidence of the different types of proteinuria in 43 cases of paraproteinaemia

	Cases	Per cent
BJPU	16	37
BJPU + albuminuria	6	14
Albuminuria	4	9
No proteinuria	17	40
Total	43	100

TABLE V Incidence of BJPU in the different types of paraproteinaemia

Type of paraproteinaemia	No of cases	No of cases with BJPU
G	20	6
A	12	5
γM	1	1
γμ	10	10
Total	43	22

positive and was otherwise positive in only one third of the cases thus it proved to be the least reliable method

In 16 cases with BJPU nine different methods for demonstrating the presence of proteinuria and BJPU were performed (table III)

Routine use of Heller's test was supplemented by precipitation with 20 % sulphosalicylic acid The presence of BJPU as estimated by paper or immune electrophoresis was never demonstrated when the sulphosalicylic acid precipitation test was negative In one of the 43 cases however BJPU could be demonstrated where Heller's test was negative

Due to the presence of mixed proteinuria, Philippen's test was negative in four cases Jacobson and Milner's test was negative in only one of these cases A negative result would have been expected in all the four cases, since a marked albumin peak was present on the electrophoretic diagram

All the four cases with mixed proteinuria suffered from complicating kidney conditions nephrocalcinosis, amyloid kidney, pyelonephritis and diabetic nephropathy, respectively

In three instances false negative Philippen's tests were turned into positive tests by adding 3 ml of the sodium chloride solution instead of the prescribed 1 ml

Table IV shows the incidence and type of proteinuria in 43 cases of paraproteinaemia

Twenty two cases (51 %) had BJPU Table V gives the incidence of BJPU in relation to the different serum paraproteins in the 43 cases

It should be noted that BJPU was demonstrated in every case of microgammamyeloma

Discussion

In earlier studies on myelomatosis the incidence of BJPU, with the boiling test as a criterion was found to vary between 8 and 87 % (usually about 50 %) (8) These widely varying results can probably be blamed on the method of examination The results may also have been influenced by the composition of the material since the diagnosis was probably made at a later stage of the disease than is customary today

In early stages of the disease BJPU

occurs intermittently and low concentrations will not be revealed by the boiling test In later stages BJPU is demonstrated more frequently and whenever it occurs it is regarded as a serious prognostic sign (15)

High concentrations of BJP may influence the heat solubility and give a negative reaction (16)

A negative Heller s test does not exclude BJPU Drivsholm (2) demonstrated by paper electrophoresis the presence of BJPU in 30 % of his cases, where Heller s test had been negative

As a screening test, we adopted the sulphosalicylic acid precipitation test, which is more sensitive than Heller s test (12) According to Phlippen a negative sulphosalicylic acid precipitation excludes BJPU This accords with our experience

It should be noted that Albustix is unreliable when proteinuria is due to BJP (2 7)

It has been claimed that a negative HCl precipitation test excludes BJPU (4) This is contrary to our experience The test is non specific and difficult to evaluate A high concentration of ordinary protein may give a positive result (6)

Efffersoe and Tidström (3) reported 90 % positivity when BJPU was present using their salting out test In our study we have found the test of little value

Jacobson and Milner s boiling test (16) may turn out positive in mixed proteinuria if the precipitate is centrifuged redissolved in normal urine and examined in the usual way This procedure we did not use The way in which we performed the test gave both false

TABLE VI Number of cases (reported by others) demonstrating the presence BJPU as evaluated by paper electrophoresis and by the boiling test

	Electrophoresis	Boiling test
Putnam and Stelos 1953	18	9
Osserman and Lawlor 1955	24	16
Riva 1957	10	8
Broch and Brodwall 1958	12	6
Phlippen 1961	12	12

positive and negative results and we found the test complicated and time consuming

Among the routine methods, we found Osgood Haskins test the least reliable According to Phlippen (13), Osgood Haskins test is unreliable when BJP is not precipitated at room temperature but only after heating or if it remains in soluble in spite of heating

Paper electrophoresis of urine is diagnostically more reliable than the boiling test According to Wuhrmann and Märki (17) a maximum of 25 % of myeloma cases show the classical heat precipitation while about 50 % demonstrate BJPU on paper electrophoresis

Several papers deal with the examination of BJPU by both paper electrophoresis and different modifications of the boiling test (1 10 12 14) Phlippen using his own test found complete agreement between these two methods while other authors found less consistency (table VI)

In Phlippen s test it is important to use sufficient salt to avoid false negative results In three of our cases it was

necessary to add 3 ml of the salt solution instead of the prescribed 1 ml Fifty cases with ordinary proteinuria were examined with this test, and none gave a false positive result

Riva (14) first demonstrated that BJP is not always heat soluble He examined ten cases with BJPU by means of the boiling test, paper- and immune electrophoresis and ultracentrifugation The boiling test dissolved the precipitate almost completely in five, partially in three and not at all in two cases

Philippen studied 12 cases, all of which had BJPU The boiling test showed complete dissolution in four, partial dissolution in six and insoluble BJP in two cases (12) Heat insoluble BJP was not found among our cases

BJPU can be demonstrated by paper electrophoresis when the protein is heat insoluble and when mixed proteinuria masks the heat solubility (14) Concentration of a small amount of albumin by means of ultrafiltration may be demonstrated as a very small peak in the electrophoresis diagram This may be of no pathological significance (16)

Since myelomatosis is often complicated by affection of the kidneys (myeloma kidney, pyelonephritis nephrocalcinosis and amyloid kidney) mixed proteinuria is likely to occur in some cases Van Dommelen collected 541 cases of myelomatosis from the literature and found BJPU alone present in 34 %, albuminuria alone in 25 % and mixed proteinuria in 6 % of the cases (16)

Immune electrophoresis has also in our work proved a reliable method for demonstrating the presence of BJPU

All types of serum paraproteins may occur in myelomatosis, all types may show BJPU, and BJPU is almost always present in microgammamyeloma Since the serum protein pattern may show no abnormality in microgammamyeloma the demonstration of BJPU is of great diagnostic significance (11, 13, 14)

The diagnosis of myelomatosis may be established at an early stage, provided that all cases of unexplained proteinuria are examined for BJPU In this respect we consider Philippen's combined sulphosalicylic acid and boiling test very useful because of its simplicity

Summary

Fourty two cases of myelomatosis and one case of macroglobulinaemia Waldenstrom were tested by different methods in order to find the most suitable method for demonstrating BJPU

For practical clinical work, Philippen's combined sulphosalicylic acid and boiling test is a simple and reliable method although negative results may be found in mixed proteinuria

Paper and immune electrophoretic examinations are the most reliable methods, since they also may demonstrate heat insoluble BJP, as well as BJP as part of mixed proteinuria

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Plasma Protein and Lipid Pattern in the Nephrotic Syndrome

By

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The classical definition of the nephrotic syndrome includes the following symptoms massive proteinuria, hypoalbuminaemia hypercholesterolaemia and oedema, with intact kidney function and normal blood pressure Nowadays most investigators agree that a glomerulopathy varying in aetiology and rendering the glomeruli more permeable to plasma proteins is the primary disturbance in the nephrotic syndrome Thus it is now reckoned that the diagnosis of a nephrotic syndrome should depend only on the magnitude of the proteinuria (21) and that hypoalbuminaemia hypercholesterolaemia, oedema reduced kidney function and hypertension may be present in varying degrees

Where the nephrotic syndrome is *known to have a cause such as chronic* glomerulonephritis amyloidosis diabetic intercapillary glomerulosclerosis poisoning etc the syndrome is often referred to as a secondary one whereas with unknown aetiology the designation as primary nephrotic syndrome is applied Cases of primary nephrotic syndrome usually fulfil the classical criteria where as reduced kidney function and hyper tension are often encountered in the secondary nephrotic syndrome

The present aim is to determine the concentration of different plasma proteins and lipids in the serum of patients with the nephrotic syndrome to investigate whether variations may be of diagnostic guidance in distinguishing nephrotic syndromes of primary and secondary type

Material

Fifty three patients with an untreated nephrotic syndrome 17 females and 36 males were examined The age ranged from 14 to 70 Twenty nine cases had a primary nephrotic syndrome (verified by kidney biopsy in 22 cases and by autopsy in 2) The remaining 24 had a secondary nephrotic syndrome verified by kidney biopsy in 16 cases and by autopsy in 4 The secondary nephrotic syndromes were due to chronic glomerulonephritis in 8 cases secondary amyloidosis in 6 pregnancy in 2 renal vein

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TABLE I. Mean values and observed ranges of various laboratory tests in 53 adult nephrotic

Aetiology	No	Serum concentration (g/100 ml)		
		TP (6.60—8.20)	ALB (4.44—5.86)	α_1 (0.23—0.43)
Primary nephrotic syndrome	29	4.37 3.37—5.50	1.62 0.70—2.90	0.28 0.17—0.46
Secondary nephrotic syndrome	24	4.83 3.70—5.79	1.99 0.46—4.19	0.32 0.25—0.44
Chronic glomerulonephritis	8	5.18 3.80—6.35	2.91 1.53—4.19	0.31 0.25—0.43
Secondary amyloidosis	6	4.72 4.00—5.79	1.35 0.46—2.50	0.34 0.29—0.44
Nephrotic syndrome in pregnancy	2	4.70 3.80—5.60	1.87 1.08—2.66	0.32 0.29—0.34
Diabetic nephropathy	2	5.99 5.48—6.49	2.44 1.45—3.42	0.38 0.37—0.38
Renal vein thrombosis	2	4.47 4.13—4.80	1.46 1.11—1.80	0.30 —
Poisoning (lead and gold)	2	3.70 3.70—3.70	0.96 0.83—1.09	0.32 0.25—0.38
Perkoff's disease	1	4.52	2.35	0.30
Hodgkin's disease	1	4.00	0.66	0.49

thrombosis in 2, poisoning with gold and lead in 2, Perkoff's disease in 1 and Hodgkin's disease in 1 case. In the last case the nephrotic syndrome was probably caused by compression of the renal vein by enlarged retroperitoneal lymph nodes since renal biopsy was normal and since radiotherapy of the abdomen led to a significant reduction in proteinuria.

Methods

Proteinuria was determined by Kjeldahl analysis of a urinary trichloroacetic acid precipitate in 26 cases and by the biuret or Shevsky and Stafford method in the remaining 27 cases. The value given in g/24 hrs is the average of from 10 to 25 days.

Paper electrophoresis was performed by a modification of the method of Laurell et al (16). The protein fractions were determined spectrophotometrically by elution of paper electrophoretic strips stained with amido black. No correction was made for trailing or different stainability of the protein fractions.

Total protein concentration was determined by the biuret method.

Serum iron was measured spectrophotometrically by the method of Luhr (6).

Transferrin in serum was determined by saturating it with excess iron. Unbound iron was removed by chromatography on an aluminium oxide column and total iron binding capacity (TIBC) was measured as serum iron (15).

Ceruloplasmin was determined after Ravin (19) and serum copper after Gubler et al (9) (Medicinsk laboratorium, Copenhagen).

Cholinesterase was determined electrometrically by the method of Tammelin (25).

Fibrinogen was measured by a modification of the method given by Jacobsson (11).

patients

			Serum cholesterol (mg/100 ml)	Serum creatinine (mg/100 ml)	Proteinuria (g 24 hrs)
α (0 31—0 59)	β (0 47—0 79)	γ (0 62—1 10)	(150—300)	(<1 3)	(100 mg)
1 00	0 76	0 74	559	1 2	9 1
0 47—1 75	0 47—1 26	0 37—1 30	250—940	0 7—2 4	3 5—19 4
0 93	0 73	0 81	362	2 1	7 8
0 64—2 17	0 40— 1 08	0 38—1 70	184—700	0 4—11 2	3 4—16 6
0 73	0 60	0 63	357	2 9	7 2
0 64—0 80	0 40—0 72	0 38—1 07	245—484	0 8—11 2	3 4—16 6
1 21	0 77	1 07	349	3 1	7 7
0 78—2 17	0 63—0 84	0 43—1 70	184—654	1 8—9 9	5 5—12 0
1 01	0 75	0 78	514	0 8	5 9
0 98—1 03	0 66—0 84	0 75—0 80	510—517	0 7—0 9	3 7—8 1
1 25	0 96	0 98	356	1 2	4 1
0 91—1 58	0 83—1 08	0 96—0 99	231—480	0 8—1 6	3 5—4 7
1 23	0 70	0 69	433	1 1	13 9
0 92—1 54	—	0 42—0 96	433	1 0—1 ?	13 0—14 8
0 83	0 94	0 68	653	1 0	10 0
0 69—0 96	0 82—1 05	0 63—0 72	606—700	1 0—1 0	8 0—12 0
0 82	0 57	0 47	279	0 9	6 0
0 99	0 76	1 10	—	0 4	7 0

Lipids Total lipids were determined by measuring the content of ester bonds (7) Phospholipids and cholesterol were extracted and determined by the methods of Fiske and Subbarow (5) and Sperry (23) respectively Non-esterified cholesterol was determined after precipitation with digitonin

Results

Table I gives the *mean value and* range for the different plasma protein fractions obtained by electrophoresis and for serum cholesterol serum creatinine and proteinuria

In 29 patients with a primary nephrotic syndrome the average values for total serum protein and serum albumin concentration were 4 37 g/100 ml (3 37—5 50) and 1 62 g/100 ml (0 70—2 90) respectively In 24 patients with a secondary nephrotic syndrome these values were 4 83 g 100 ml 3 70—5 79 and 1 99 g 100 ml 0 46—4 19

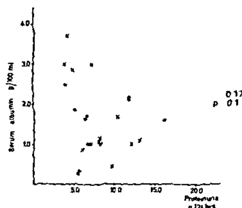


Fig 1 Relation between proteinuria and serum albumin in 53 adult nephrotic patients ● primary nephrotic syndrome × secondary nephrotic syndrome

TABLE II. Plasma proteins in the nephrotic syndrome

Protein	Molec. weight	No. of pats.	Concentration Normal	Decreased	Increased
Albumin	69 000	54	0	54	0
Transferrin	90 000	36	17	19	0
Ceruloplasmin	160 000	21	2	19	0
Cholinesterase	300 000	23	11	5	7
Fibrinogen	341 000	19	1	0	18

Fig. 1 indicates an inverse relationship between proteinuria and serum albumin concentration in the 53 patients, but the correlation is not statistically significant (r = −0.17, p > 0.1).

Serum *transferrin* and serum iron were measured in 36 nephrotic patients, 21 with a primary and 15 with a secondary syndrome. Transferrin was decreased in ten of the 21 patients with a primary nephrotic syndrome (104—221 µg/100 ml) and in nine of the 15 patients with a secondary syndrome (76—212 µg/100 ml) and was normal in the remaining 17. Saturation index (serum iron/TIBC) was lowered in 19 cases, ranging from 0.10 to 0.23 (normal range 0.26—0.49 (4)) and was normal in 17.

In 21 patients with primary nephrotic syndrome the saturation index was normal in nine and decreased in 12. In these cases haemoglobin was reduced in eight (6.4—13.8 g/100 ml) and normal in four. In the nine patients with a normal saturation index the haemoglobin varied from 10.6 to 17.6 g/100 ml.

Ceruloplasmin analysis on serum was performed in 21 patients. It was decreased in 19 and normal in two. These two patients had a primary ne

TABLE III. Mean values and ranges of different plasma lipids in 23 adult nephrotic patients

	No.	TC (m moles/l)	NFC (m moles/l)	NFC/TC
Primary nephrotic syndrome	15	13.39 (6.23—21.34)	3.22 (1.24—4.90)	0.24 (0.19—0.29)
Secondary nephrotic syndrome	8	9.01 (4.50—13.98)	2.29 (1.13—3.30)	0.25 (0.21—0.30)
Normal range		3.85 [illegible] 25	0.82—1.62	0.20—0.24

TC = total cholesterol
PL = phospholipids
FA = fatty acids
NFC = non-esterified cholesterol
TEFA = total esterified fatty acids
TC = triglycerides

phrotic syndrome and, respectively, a proteinuria of 3 5 and 14 6 g/24 hrs Serum copper was measured in ten cases It was normal in nine and slightly lowered in one, 60 μg/100 ml (normal range 85—145)

Cholinesterase was determined in 14 patients with a primary and in nine with a secondary nephrotic syndrome In the first group it was normal in eight, increased in five and decreased in one In the nine cases with a secondary syndrome it was normal in three increased in two and decreased in the remaining four

Fibrinogen was measured in 19 patients It was elevated in 18 and normal in one This patient was a 60 year old man with a primary nephrotic syndrome and a proteinuria of 9 8 g/24 hrs A significant correlation ($r = 0\ 71$, $p < 0\ 001$) was found between α_2-globulin and fibrinogen in these 19 patients (fig 2) The results are summarized in table II

Lipids Total serum cholesterol was measured in 29 patients with a primary nephrotic syndrome and in 20 with a secondary syndrome It was elevated in

PL (m moles l)	TEFA (mEq/l)	FA in TC (mEq/l)
5 12	26 9	7
(3 12 –8 34)	(16 0—33 7)	(2—22)
3 87	20 4	6
(2 16—5 18)	(9 2—32 1)	(2—15)
2 00—3 45	8 0—14 3	<4

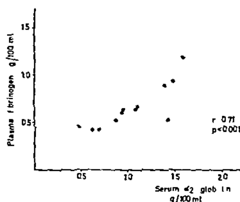


Fig 2 α_2 globulin and fibrinogen in 19 adult nephrotic patients

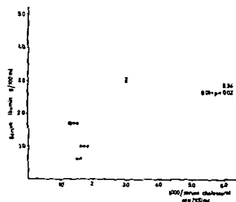


Fig 3 Relation between serum albumin and the reciprocal serum cholesterol in 49 adult nephrotic patients ● primary nephrotic syndrome × secondary nephrotic syndrome

28 and 15 cases respectively and normal in the remaining six Fig 3 illustrates the relation between serum albumin and the reciprocal value of serum cholesterol in the 49 patients ($r = 0\ 34$ $0\ 01 < p < 0\ 02$) Fractionated serum lipids (triglycerides phospholipids total cholesterol and non esterified cholesterol) were determined in 23 patients 15 with a primary and eight with a secondary nephrotic syndrome

The ratio of non esterified to total cholesterol was elevated in seven of the

patients with a primary and in five with a secondary nephrotic syndrome. In the remaining 11 patients it was normal.

Phospholipids were elevated in 14 and five cases and triglycerides in nine and four cases in the two groups. The remaining cases had a normal concentration of these two lipids. Only in one patient, a 65-year-old man with secondary amyloidosis and a proteinuria of 6.0 g/24 hrs, the plasma lipid pattern was normal. The results are summarized in table III.

Discussion

The concentration of a specific plasma protein is the net result of a number of variables: synthesis, degradation, external loss and plasma volume. Only turnover studies with labelled plasma proteins can reveal which of the above mentioned processes are involved, but such studies have been done only with certain of the plasma proteins (1, 12, 13, 14).

The well-known electrophoretic pattern of nephrotic sera (21) was also found in this study.

In the total material *serum albumin* was depressed in 100 %, α_2-globulin elevated in 95 % and β-globulin in 30 %.

The different groups of secondary nephrotic syndrome are too small to allow any conclusions as regards differences in the various protein fractions. In the two main groups, primary and secondary nephrotic syndrome, the only significant difference observed was that serum albumin was 23 % higher in the secondary group. This might be explained by difference in albuminuria. Proteinuria was on an average 17 % higher in patients with a primary nephrotic syndrome.

Fig. 1, which shows the relation between proteinuria and serum albumin in the whole material, indicates that in general an inverse relationship existed between proteinuria and serum albumin. However, no significant correlation was found between these two parameters. This implies that other factors (synthesis, endogenous degradation and plasma volume) influence the serum albumin concentration. Both the rate of synthesis and the endogenous degradation of albumin have been shown to be elevated in several cases of nephrotic syndrome (13).

The paper electrophoretic *gamma component* is composed of the immunoglobulins which comprise IgG, IgA and IgM. McKelvey and Fahey (18) found a decreased concentration of IgG and IgA in nephrosis, whereas the concentration of IgM was increased. Turnover studies in the nephrotic syndrome have so far been performed with only one of the immunoglobulins, namely IgG. In some cases an internal hypercatabolism contributed to the lowered serum concentration, accentuating the effect of urinary losses (1).

In this study the average γ-globulin concentration was 0.74 g/100 ml (0.37 – 1.30) in the group with a primary nephrotic syndrome and 0.81 g/100 ml (0.38 – 1.70) in the group with a secondary syndrome. The highest value was observed in the six patients with amyloidosis, average 1.07 g/100 ml (0.43 – 1.70), and in the patient with Hodgkin's disease: 1.10 g/100 ml.

Among 29 patients with a primary

nephrotic syndrome the concentration of γ globulin in serum was decreased in 13, normal in 14 and increased in two In 24 cases of secondary nephrosis serum γ globulin was decreased in seven, normal in 13 and increased in four

In agreement with Schreiner (21) this study emphasizes that the electrophoretic pattern of the plasma proteins is of little value in assessing the aetiology of the nephrotic syndrome

Transferrin has a molecular weight about 90 000 and is lost in the urine in nephrosis (14) Besides this renal loss an endogenous degradation has been found in several cases (14) Nevertheless the serum concentration was normal in about half the patients in this study Presumably this is caused by an increased synthesis Among fifteen patients with protein losing gastroenteropathy and eight patients with nephrosis Jensen et al (14) found an increased synthesis of transferrin in nine and seven respectively

Ceruloplasmin the copper binding protein of plasma, was determined in 21 patients and found to be decreased in 19 and normal in two No turnover studies with ceruloplasmin in the nephrotic syndrome have been published Ceruloplasmin has a molecular weight of 160 000 and the decreased serum concentration also observed by other investigators (17) is presumably due to a combination of renal loss (4) and endogenous hypercatabolism as for albumin transferrin and IgG, but further work is needed

The enzyme *cholinesterase* is an α_2-globulin with a molecular weight of 300 000 It is synthetized in the liver and its serum concentration is found to be related to the hepatic synthesis of albumin (10) In this study the enzyme was elevated in eight of 24 cases which agrees well with the accelerated albumin synthesis found in several cases of the nephrotic syndrome (13) In cases with a normal or decreased concentration of cholinesterase, renal loss and presumably endogenous hypercatabolism may balance or outweigh the synthesis

Fibrinogen was determined in 19 cases It was elevated in 18 and normal in one Its molecular weight is about 400,000 It is not lost in the urine in the nephrotic syndrome

α macroglobulin is known to be a plasmin inhibitor (24) Jacobsson (11) found an increased plasmin inhibitor activity in nephrosis In this study a highly significant correlation was found between α globulin (much of which is α macroglobulin) and fibrinogen Thus the elevation of α macroglobulin in nephrosis (24) might be responsible for the increased antiplasmin activity and hence an augmented fibrinogen concentration as suggested by Jacobsson (11)

The *hyperlipaemia* seen in most cases of the nephrotic syndrome is due to a rise in lipoproteins Therefore it would be more correct to talk of hyperlipoproteinaemia than of hyperlipaemia

The concentrations of the different lipid fractions (triglycerides cholesterol and phospholipids) have been found to depend on the severity of the nephrotic syndrome (2) In mild and moderate cases serum cholesterol and phospholipids were elevated whereas the triglycerides were normal In severe cases all fractions were increased

In this study ten patients with a normal plasma concentration of triglycerides had an average proteinuria of 7.5 g/24 hrs (3.4—16.5) and 13 patients with increased triglycerides had an average proteinuria of 10.0 g/24 hrs (3.5—19.4). This does not support the above mentioned observation of Baxter et al. (2), but the present material is too small to allow any definite conclusion. In severe but not in milder cases the elevation of cholesterol and phospholipids might be secondary to the increased concentration of triglycerides.

The hypoalbuminaemia has been claimed to contribute to the pathogenesis of the hypertriglyceridaemia (2, 3). Baxter et al. (2) found an inverse linear relationship between serum albumin and serum cholesterol, phospholipids and triglycerides. In this study no significant correlation could be found between serum albumin and the reciprocal serum cholesterol (fig. 3). Moreover serum cholesterol was normal in one patient with a primary and in five patients with a secondary nephrotic syndrome in agreement with Shearn (22) who gathered from the literature 15 cases of LED nephrosis with normal serum cholesterol.

Here is not the place to elaborate present day views on the removal of triglycerides from the blood stream. From several investigations the following hypothesis is plausible: lipoproteins synthetized in the intestinal epithelium or in the liver are transferred to the blood. Here the lipid moiety of lipoproteins is split by a lipoprotein lipase into free fatty acids (FFA) and glycerol, and FFA are transported by serum albumin (20). As regards the protein moiety the very low density lipoproteins (chylomicrons) are presumably transformed into low density lipoproteins (density 1.019—1.063).

By use of radioiodinated lipoproteins Gitlin et al. (8) found a decreased conversion of lipoproteins with a density below 1.019 into lipoproteins with a density from 1.019 to 1.063 in childhood nephrosis. Their results suggest a disturbance in the lipolytic process. The low serum albumin may contribute to this disturbance, but is not the whole explanation.

Lipoprotein lipase attacks only triglycerides. Thus in cases of nephrosis where only cholesterol and phospholipids are elevated (vide supra) no explanation is yet available for these elevations.

Low density lipoproteins have an electrophoretic mobility as for β globulins, and very low density lipoproteins as for α globulin. Thus the hyperlipoproteinaemia contributes to the elevation of these globulins in the nephrotic syndrome. However, the elevation of α_2 globulin also reflects an increased concentration of α_2 macroglobulin (24). As yet there is no explanation for the elevation of α_2 macroglobulin in the nephrotic syndrome. This must await turnover studies with this plasma protein.

Summary

Paper electrophoresis on serum was performed in 53 adult nephrotic patients of whom 29 had a primary and 24 a secondary nephrotic syndrome. In some patients the specific globulin fractions

transferrin (36 cases), ceruloplasmin (21) cholinesterase (23), and fibrinogen (19), were also determined Furthermore serum cholesterol was measured in 49 and fractionated serum lipids in 23 cases

It is concluded that neither the electrophoretic pattern of serum nor the concentration of the other proteins and lipids examined is of any value in assessing the aetiology of the nephrotic syndrome

Possible and verified explanations of the altered concentration of the different plasma proteins and lipids in the nephrotic syndrome are discussed

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From the University Lung Clinic (Head Gosta Birath M D) Renstromska sjukhuset Goteborg and the Institute of Pathology, University of Goteborg Goteborg Sweden

Idiopathic Interstitial Fibrosis of the Lungs

III Pathology and serology

By

Lars Ander[1] and Lennart Zettergren

In previous papers, a group of 19 patients with idiopathic interstitial fibrosis of the lungs (IFL) was analysed for prognosis as indicated by radiological findings (2) and reversibility of respiratory disturbances during steroid treatment (18)

The present study concerns the pathoanatomical changes in the 14 cases in which biopsy and or autopsy specimens were examined Special attention is devoted to the association between pathoanatomical and roentgen findings and to the relationship between the appearance of the lungs in the biopsy material and steroid induced reversal of the changes

The etiology of IFL is unknown Several authors have suggested that IFL is of rheumatoid origin (19 23 24 26) or an immunopathy (15 17 22) To elucidate the etiology serological and immunological examinations were made in 14 of the 19 cases

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Material

The patients were described in the previous papers (2 18) with the same case numbers as now Seven of the 19 patients underwent diagnostic thoracotomy with lung biopsy (nos 3 7 10 11 13 17 19) and autopsy was performed in eight of the nine fatal cases (nos 1 5 6 8 12 13 14 18) In one of the autopsy cases (no 13) diagnostic thoracotomy had been carried out before the start of steroid therapy six months before death table I)

Methods

At *thoracotomy* the gross appearance of the lung its consistency and ventilatory resistance were appraised *Biopsy* was attempted from both the apparently non diseased and the diseased parts Peripheral regions of the lingular intermediate and inferior lobes were avoided as they form part of the most poorly ventilated sections of the lung (7 Specimens were pinched off in the insufflated state and immediately fixed Specific and non specific bacteria and fungi were cultivated

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Five of the *autopsy records* (nos 1, 5 6 8 and 12) were obtained from the hospital files The remaining three patients died during the present investigation and were given special attention at the autopsy In no 14, one lung was examined according to the large section method devised by Gough et al (8) Lung tissue for micro scopic examination was paraffin embedded sectioned and stained with hematoxylin—van Gieson hematoxylin eosin resorcin fuchsin according to Weigert periodic acid Schiff (PAS) and silver impregnation according to Wilder

Serological examination included the Sheep Cell Agglutination Test (SCAT), Anti Streptolysin Titer (AST) serum elec trophororesis, LE cell tests (at least three) and Wasserman s Reaction (WR)

In six cases, diffusion in gel analysis was carried out at the Department of Bacteri ology University of Goteborg by Dr Stig B Holm Antigen was extracted from healthy human lung tissue obtained at operations for diaphragmatic hernia the comparative double diffusion in gel tech nique of Ouchterlony (21) was employed in a micro modification described by Wads worth (27) All sera were tested against the lung antigen at various dilutions

Case reports

Four cases were selected to demonstrate different clinical courses and varying effects of corticosteroid treatment

Case 10

Forty eight year old man Welder at ship yard since age 27 Treated for prostatitis operated on for gall stone No joint symp toms

Routine roentgen check up in 1955 in view of his occupation discrete pin point shad ows as in welder s lung stationary at sub sequent check ups until 1960

From early 1961 increasing shortness of breath coughing blood tinged sputum fever After rapid deterioration admitted to the Lung Clinic three months after onset of symptoms

On admission Temp 38° C Pulse 90—108/min B P 145/65 mm Hg Resp rate 18—36/min Pronounced dyspnea marked cyanosis Clubbed fingers Fine rales in both lungs

Hb 15 6 g% White cell count 6 700 ESR 65 mm/hr

Lung roentgen Mottling and partly confluent patchy clouding with poorly de fined margins in lower halves of both lung areas (fig 1a) No pleural changes or hilar enlargement

Pulmonary function VC 56 % of calc normal value S_aO_2 at rest 77 % P_aO_2 42 mm Hg $P_{A-a}O_2$ 80 mm Hg P_aCO_2 25 mm Hg on air breathing

ECG No signs of cor pulmonale

Thoracotomy and biopsy after about two months of corticosteroid therapy The lungs showed granular, firm lower and inter mediate lobes but relatively normal upper lobes

Microscopic examination Pulmonary parenchyma showed varying degrees of fibrosis partly in small bands partly dif fusely as thickened alveolar walls Abundant proliferation of fibroblasts and infiltration of round cells are seen in these walls (fig 2) Also the alveolar epithelium displays moderate proliferation In the interstitial tissue there are sparse, powder fine in crustations with iron rich pigment PAD Siderosis pulm (welder s lung) incipient idiopathic interstitial lung fibrosis?

Initially unspecific pneumonia or pul monary emboli were suspected and anti biotics and heparin were administered for one week without effect With prednisolone therapy initially 40 mg swift regression on the roentgen film almost to pre morbid appearance (fig 1b), rapid subjective im provement almost normal blood gas values increase in vital capacity and normal pulse rate On reduction of the corticosteroid dosage perhaps too hastily roentgenologic progression without subjective or functional deterioration On increased dosage previous roentgenologic improvement was regained



Fig 1a Case 10 Maximal mottling and poorly demarcated patchy clouding in the lower half of the lungs



Fig 1b Case 10 Optimal improvement after corticosteroid therapy

Comment

Acute form of IFL Good improvement during corticosteroid treatment which was continued for more than three years

Case 14

Fifty three year old man Worked in cotton mill from 11 to 25 years of age thereafter taxi driver European wrestling champion at age 25 At times considerable consumption of alcohol

About 1950 he noticed increased shortness of breath on exertion but was able to carry out his work In 1951 normal lung roentgen Sought treatment in 1962 because of more pronounced exertion dyspnea and dry cough No fever or loss of weight.

On admission in 1962 Temp 36 9° C Pulse 96/min B P 145/90 mm Hg No cyanosis or dyspnea at rest on first examination but these developed later to a pronounced degree Obvious clubbed fingers Normal heart sounds and size Fine rales basally in the lungs

Hb 17 0 g% White-cell count 4 700 ESR 6 mm/hr

Lung roentgen Bilaterally thin ring contours, mostly located sub-pleurally (fig 3a Later this honeycomb pattern became general, the diaphragm was elevated and

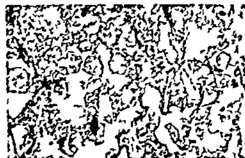


Fig 2 Case 10 Lung tissue with moderate fibrosis and sparse round-cell infiltration Biopsy after three months treatment with prednisolone Good effect H E van Gieson

increased filling of the pulmonary arteries centrally was also observed On bronchography Peripherally deformed club-shaped raised bronchi with centrally straightened course The usual contrast free subpleural zone was lacking (fig 3b)

Pulmonary function VC 62 % of predicted value S_aO_2 light work and air) 93 % P_aO_2 70 mm Hg $P_{A-a}O_2$ 39 mm Hg P_aCO_2 37 mm Hg

ECG Pattern as in right ventricular strain

Microscopic examination of mediastinal lymph nodes showed chronic lymphadenitis



Fig 3a Case 14 Lung roentgen Details of left upper lobe showing honeycomb pattern most pronounced subpleurally



Fig 3b Case 14 Details of bronchogram of right upper lobe Bronchi in their proximal region stretched peripherally subpleurally clustered together with cyst formations as indicated without subpleural contrast free zone

Corticosteroid medication started after about one year's observation Despite this gradual deterioration with very pronounced dyspnea Died six months later

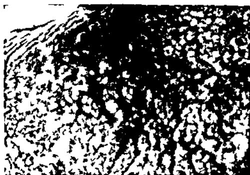


Fig 4a Case 14 Part of lung with fine lobed pleural surface



Fig 4b Case 14 Section from lung in fig 4a Fibrosis with numerous small cystic structures

Autopsy Right heart hypertrophic Pulmonary arteries free Lungs considerably diminished in volume notably firm generally of hobnail cirrhotic appearance with small cysts On the cut surfaces strongly fibrosed parenchyma interspersed with small cystic changes (figs 4a and 4b)

Microscopic examination The lung parenchyma shows considerable fibrosis with widespread round cell infiltration In some regions the alveolar pattern is almost entirely destroyed and there are groups of small cystic alveolar ducts and respiratory bronchi lined with atypical dark celled columnar epithelium In other regions the alveolar pattern is preserved with moderately thickened alveolar walls The walls of the larger bronchi are reconstituted Both alveolar and bronchial epithelium show squamous metaplasia The

vascular walls are fibrosed Inflammatory exudation in some alveoli PAD Idiopathic interstitial lung fibrosis

Comment

Case of IFL with widespread bilateral lung roentgen changes dominated by honeycomb pattern already at the first examination Swift deterioration and death in spite of corticosteroid medication

Case 18

Fifty nine year old male cobbler Angina pectoris since about 1950 Suspected lung tbc in 1926, lung roentgen films not available

Increasing shortness of breath and cough with colourless expectoration since about 1950 On lung roentgen film in 1956 minimal pin point shadows supraclavicularly Admitted in 1963 because of increasing deterioration and loss of weight during ten months

On admission Temp 36 4° C Pulse 86/min B P 140/70 mm Hg Dyspnea at rest and cyanosis No clubbed fingers Normal physical findings from heart Fine rales basally in the lungs

Hb 13 3 g% White cell count 6 500 ESR 29 mm/hr

Mantoux 0 05 mg pos 15 × 15 mm infiltration Repeated Lowenstein cultivations and guinea pig tests for Mycobacteria neg

Lung roentgen Bilateral lesions in form of apical ring shadows of various sizes partly of honeycomb pattern partly several centimeters in diameter Also basally and subpleurally similar changes with progressive elevation of the diaphragm and dislocation to the right of the trachea (fig 5)

Pulmonary function VC 38 % of predicted value S_aO (at rest and air breathing) 83 % P_aCO_2 32 mm Hg Agonal increase of P_aCO_2 to 56 mm Hg

ECG Right sided bundle branch block

Initially tuberculosis was suspected



Fig 5 Case 18 Bilateral apical preponderance of lung lesions with various size cysts and subpleural honeycomb pattern Elevation of the diaphragm dislocation of the trachea to the right

Treated with chemotherapeutics and antibiotics but without effect Repeated negative bacteriological examinations and progressive deterioration during treatment made us consider sarcoidosis instead Corticosteroid medication for three months without effect Ad mortem with appearance of secondary unspecific bronchopneumonia

Autopsy Heart uniformly enlarged Old myocardial infarction in anterior wall of left ventricle coronary atheromatosis Pleural adhesions on right side Lungs small with increased firmness surface coarsely granulated Bands of fibrosis on cut surfaces in between millet sized vacuoles

Microscopic examination Lung parenchyma has varying appearance with strongly fibrosed areas and a number of small cystic structures Moderate round cell infiltration in connective tissue Alveoli with slightly or moderately thickened walls in areas Here and there squamous metaplasia of the proliferating bronchial epithelium In other regions the lung parenchyma is not particularly fibrosed but the alveoli contain inflammatory exudation with a few red blood corpuscles lymphocytes polymorphonuclear leukocytes and desquamated alveolar epithelium In some of these alveoli hyaline membranes are seen (fig 6) Moderately pronounced chronic

TABLE I Macroscopic and roentgenologic findings[1]

Case	Sex	Age at onset	Thoracotomy	Autopsy	Gross appearance	Increase in firmness or resistance
1	♀	41		x	Nodular small cysts	x
3	♂	51	x		Normal	x
5	♂	67		x	White nodules one cm cysts	x
6	♂	73		x	White nodules emphys cysts pur inf	x
7	♂	64	x		Antracot nodules	Normal
8	♀	87		x	Large greyish red	x
10	♂	48	x		Nodular	x
11	♂	65	x		Superficial nodules	No information
12	♀	81		x	Uniformly nodular	x
13	♂	60	x	x	Uniformly cystic nodular	x
14	♂	53		x	Cirrhotic small cysts	x
17	♀	53	x		Nodular	x
18	♂	59		x	Cysts of varying size	x
19	♀	49	x		Cirrhotic	x

[1] Investigations carried out and features present are denoted x Feature not found 0 findings uncertain investigations not carried out —

bronchitis and peribronchitis Intimal fibrosis and in places, intramural round cell infiltration in the vessels PAD Idiopathic interstitial lung fibrosis

Comment

Chronic IFL with initially minimal apical changes Lung roentgen films taken during seven years showed increasing concentration of lesions in the upper parts of both lungs and development of basal and subpleural lesions too The lung roentgen films suggested strongly lung tuberculosis and IFL was not considered during life

Case 19

Forty nine year old housewife with history of shortness of breath, coughing with scanty serous sputum pains in the chest and swallowing difficulties since 1956—57 Normal lung roentgen film one year before symptoms Erythema nodosum on rheumatic basis 24 years before onset of disease Never any joint trouble

On admission in 1963 Temp 36 9° C Pulse 78/min Respiration rate 15/min B P 185/105 mm Hg No dyspnea at rest no cyanosis No clubbed fingers Normal heart sounds Lungs Fine râles basally and dorsally

Hb 14 5 g% White cell count 6 000 ESR 21 mm/hr

Lung roentgen Basally a faint haze with a fine granular appearance Increasing lesions with a diminished volume of the basal parts of the lower lobes Sparse honeycomb pattern The changes were observed on the lateral view as a band shaped density above the diaphragm, composed of

Extent of gross lesions compared with roentgen appearance		Honeycomb lesions		Cor pulmon at autopsy	Steroid therapy
Bigger than X ray	Same	At roentgen film	At thoracotomy or autopsy		
x		x	x	x	
	x	0	0	—	x
	x	x	x	x	
	x	x	x	0	
No information	No information	0	0	—	x
	x	x	?	?	x
x		0	0	—	x
x		0	?	—	x
	x	x	x	0	x
	x	x	x	x	x
	x	x	x	x	x
x		0	?	—	x
	x	x	x	0	x
x		x	x	—	x

minor ring contours (fig 7) Bronchography confirmed the shrinkage of the basal parts of the lower lobes with reduction of the usually unfilled subpleural area

Pulmonary function VC 82 % of predicted value S_aO_2 (at light work and air) 96 % P_aO_2 94 mm Hg P_aCO_2 44 mm Hg $P_{A-a}O_2$ 9 mm Hg

LCC Normal findings

AST 800 units/ml SCAT 1/20

Thoracotomy Obvious fibrotic changes The lower lobe except for the apical segment the lingular lobe and the anterior segment of the upper lobe were diminished in volume and increased in firmness The surface was cirrhotic Distribution more extensive than shown by the roentgen film

Microscopic examination Lung parenchyma shows pronounced fibrosis with partly destroyed alveolar pattern Fairly large number of inflammatory cells in the connective tissue mostly lymphocytes and plasma cells but also polymorphonuclear leukocytes The connective tissue with sparse cell population and small cystic structures coated with cubical epithelium In other parts of the lung parenchyma better preserved alveolar pattern with thick fibrous alveolar walls infiltrated with round cells In all bronchi squamous metaplasia PAD Idiopathic interstitial lung fibrosis

Corticosteroid treatment for six months without roentgenologic or clinical effect

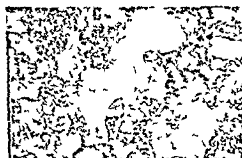


Fig 6 Case 18 Inflamed lung tissue with a couple of alveoli coated with hyaline membranes H E van Gieson

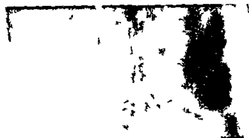


Fig 7 Case 19 Lateral tomogram detail from base of left lung Band like pattern above the diaphragm with hypertransluscent ring shadows and diminished volume of the lower lobe



Fig 8 Case 1 Lung with only slight changes in the form of fibrous thickening of the alveolar walls Wilder s silver impregnation

Comment

Chronic IFL with typical basal location Extent at thoracotomy greater than on the roentgen film No improvement with corticosteroids

Pathology

Gross appearance of the lungs (table I)

Granular, small cystic changes were observed on the surfaces of the lungs at thoracotomy and or autopsy in 11 cases *Increased firmness* was noted in 12 cases at thoracotomy or autopsy Increased *respiratory resistance existed in five* of the operated cases according to the anesthetist (information lacking in one case) Granulation and marked lobulation, combined with increased firmness caused the lung in case 14 to resemble a cirrhotic liver (fig 4a) The lungs were *considered normal in two* thoracotomy cases (nos 3 and 7)

Pronounced band or net like *fibrosis* was observed on the cut surface in all autopsy cases Numerous cysts often grouped and up to peppercorn size were seen in most cases (fig 4b) *Cysts* with a diameter of approximately one cm were observed in only two of the autopsy cases There was widespread fibrous pleurisy in three cases

Secondary bronchitis and bronchopneumonia was clinically diagnosed and confirmed in three cases and was found in two others at autopsy The hilar lymph nodes were moderately enlarged in two cases

Microscopic appearance of the lungs (table II)

Fibrosis was more pronounced in tissue obtained at autopsy than at biopsy In mild cases, the fibrosis appeared only as thickening of the alveolar walls (figs 2 and 8) In regions with pronounced fibrosis the alveoli were widely separated by a sparsely celled connective tissue with infiltration of lymphocytes and plasma cells

The *alveolar epithelium* was proliferating in the majority of cases, partly desquamated and transformed to large pale cells resembling macrophages In many cases it was partly columnar and had chromatin rich nuclei (fig 9) The alveoli in most cases contained an inflammatory exudate consisting of lymphocytes and polymorphonuclear

TABLE II Basic microscopic pulmonary changes in IFL[1]

Case no	Type of material[2]	Interstitial fibrosis	Round cell infiltration	Secondary infection	Bronchial or alveolar epithelium metaplasia	Hyaline membranes	Bone islands	Honeycomb structure	Vascular changes
1	A	++	++		+		+	++	+
3	B	+	+						
5	A	+++	++	+			+	+	+
6	A	+++	+	+++				+	+
7	B	+	+				+		
8	A	++	++						
10	B	+	+						
11	B	++	++						+
12	A	+++	+	+	+	+			
13	B+A	+++	+	+	+	+	+	+	+
14	A	+++	+	+	+			+	+
17	B	+	+						+
18	A	+++	+	+	+	+		+	+
19	B	++	+		+				+

[1] Degree of findings denoted +, ++, +++ resp

[2] A=autopsy material B=biopsy

leukocytes including eosinophils Hyaline membranes were observed in three cases (fig 6)

The *bronchiolar epithelium* presented *squamous metaplasia in six cases* In two cases the epithelium of the smaller bronchi was destroyed, and loose granulation tissue protruded into the bronchial lumen (fig 10) Bronchiolectases and small cystic dilatations of alveoli — the basis for the gross honeycomb appearance — were recorded in nine of the 14 cases (fig 11) Secondary unspecific inflammatory changes were observed in five cases PAS staining failed to disclose fungous cells in the parenchyma.

Small usually subpleural *metaplastic islets* of mature osseous tissue were encountered in four cases (fig 12)

Moderate interstitial deposition of pigment containing iron was observed in case no 10 In case no 11 a couple of small silicotic granulomata with bi-refractive particles were found in the biopsy material this patient had been a road and power plant worker (see table I in ref 2) Varying degrees of anthracosis were found in almost all cases



Fig 9 Case 1 Fibrosed lung tissue with dilated alveoli with high columnar dark celled epithelium H E van Gieson



Fig 10 Case 17 Bronchiole with destroyed epithelium and protruding granulation tissue H E van Gieson



Fig 11 Case 1 Fibrosed lung tissue with numerous small cystic structures so called honeycomb structure H E van Gieson



Fig 12 Case 13 Fibrosed lung tissue with a small islet of mature bone tissue H E van Gieson

Vascular and cardiac lesions

The pulmonary artery had in its finer branches, fibrosis in about 2 3rds of the cases The fibrosis was mostly located to the intima, which in individual vessels was sparsely infiltrated by lymphocytes

In case no 13, a massive, fresh lung embolus was found In another case atheromatosis in the pulmonary artery Hypertrophy and/or dilatation of the right heart were observed in four of the autopsy cases (table I)

Extent and appearance of pulmonary changes compared with roentgen findings

Insufficient detail in the case and operation reports make this retrospective appraisal difficult Available information is given in table I

In five cases (nos 1, 10, 11, 17 and 19) the lung changes actually were considerably more extensive than appeared from the roentgen films In eight cases there was good conformity between the gross pulmonary findings and the roentgen films, while in one case available data were insufficient for comparison

In cases where honeycomb formations were noted at operation or autopsy, comparable roentgen films have also shown them

Reversibility of pulmonary changes, judged from the microscopic pictures (table II)

The degree of fibrosis was insignificant in four of the biopsy cases (nos 3 7, 10, 17) Provided that the biopsies were representative, relatively good effects of corticosteroid treatment were therefore to be expected The roentgen findings too indicated that the results of such treatment might be good Actually, the administered steroids gave a very good result in only one case no 10 in the above case reports (figs 1a, 1b and 2)

In two cases nos 3 and 7, deterioration was halted and in case no 17 the treatment has given no improvement to date

In two biopsy cases (nos 11 and 19), the moderate degree of fibrosis on microscopy indicated that some effect of steroid treatment might be expected, but this happened initially in only one of them (no 11)

In case no 13 it was deemed from both lung biopsy and roentgen film that corticosteroid treatment was hopeless The therapeutic effect was indeed nil in this case The patient died six months after thoracotomy The pulmonary lesions observed in the autopsy material in this case were mainly identical with those in the biopsy material

Serological and immunological examinations

The *SCAT* was positive in cases nos 10 (1/80) and 11 (1/160) It showed a borderline value of 1/40 in case 13, and was negative in cases nos 3 7 9 14 15 16 17 and 19 It was not examined in the remaining eight patients None of the patients with positive reactions had suffered from arthritis Patient no 19 had had 24 years earlier, erythema nodosum which was thought to be rheumatic One of the two patients with the highest SCAT titers no 10 in the above case reports had been occupationally exposed to dust as a shipyard welder and had also been treated for prostatitis Case no 11 who also had a positive SCAT titer had likewise been exposed to dust as a tunnel worker but had no history of focal infection

The AST showed pathologic values in cases nos 19 (800), 17 (400), and 11 (200), and normal values in nos 1, 3 7 10 13, 14, 15, and 16 It was not examined in the remaining eight patients Case no 11 had both positive SCAT and positive AST Patient no 17 had suffered from rheumatic fever 15 years previously, with no persisting symptoms she had a positive AST but negative SCAT

Serum electrophoresis showed slightly abnormal patterns in cases nos 2 8 9, 10 11, 16 17 and 19 two of these (10 and 11) also had positive SCAT

In all patients with positive SCAT and or increased AST (nos 10 11, 13 17 and 19), the LE cell test was negative on at least three occasions This test was performed also on patients nos 3 4 6 7 9, 14 16 and was found negative

The *Wasserman reaction* was positive in patient no 7 who, however, showed no syphilitic changes at autopsy

Immuno precipitation with fresh sera from patients nos 2 3 7 10 11 and 13 was analysed against the lung tissue preparation with the double diffusion in-gel technique and was found negative in all these cases

Discussion

Grading of the disease and results of treatment

In assessing the response to treatment Livingstone et al (16) found it of value to grade the disease His evaluation of the alveolar changes was based mainly on microscopic appearances which correspond to those observed by the naked eye Grade I is characterized by fibrous thickening of the alveolar walls or in more acute stages by edema In grade II the alveolar spaces usually contain a

fluid exudate strongly eosinophilic, suggesting a high protein content and often with numerous alveolar macrophages Occasionally fibrin, mucus, red cells, eosinophils, or granulocytes may be seen In grade III the alveolar architecture is blurred but the bronchioli can still be recognized In grade IV the normal lung structure is effaced by fibrosis, although remnants of bronchiolar epithelium and muscle may still be recognized In grade V the lung is converted to cystic spaces of varying diameter, up to one cm or more

Grossly the lung appears normal in grade I, while in grades II and III it seems consolidated In grades IV and V the cysts and scars are easily recognized and the external surface of the lung presents a hobnail cirrhotic appearance because of the mixture of condensation, scars and expansion into cysts

In our opinion such grading on microscopic examination of the biopsy material is facilitated by inspection and palpation of the lung and estimation of the ventilatory resistance during the thoracotomy These observations supplement the information provided by the roentgen findings and thereby may influence the assessment of the reversibility and prognosis of the disease

In four cases (nos 10 11 17 and 19) the lung lesion proved to be much more widespread already at thoracotomy than was indicated by the roentgen films, there were visible lesions and considerably increased firmness in sections of the lung, with no evidence of pathological changes from the roentgen survey even at re examination Changes characteristic of IFL were also established on microscopic examination of the biopsy material

The result of corticosteroid treatment was worse than we expected from the microscopic findings alone This indicates that isolated, small biopsies do not reflect the condition of the lung as a whole

Treatment with corticosteroids suppresses the inflammatory reaction but existing fibrosis is not affected It was shown in a previous report (18) that, during corticosteroid therapy, the increased alveolar arterial oxygen pressure difference was decreased, indicating reversal of the abnormal ventilation/perfusion conditions rather than of the diffusion' impairment The improvement of the ventilation/perfusion conditions probably corresponds to grades I and II of Livingstone et al, i e edema and acute inflammatory reaction

Etiology

A number of authors have reported lung changes, identical with those in IFL in patients with rheumatoid arthritis (4, 5, 19, 23), as well as in anarthritic" rheumatic diseases with interstitial lung fibrosis (6, 20, 24, 26) Positive reactions to rheumatic factors occur in 4—5 7 % of a normal population (14, 28)

In our series, four of ten examined patients showed weak SCAT titres, two of which were appraised as positive reactions This is more than would be found in random subjects and accords with published reports (24, 26) In common with previously reported cases of IFL with positive SCAT, our patients lacked signs or histories of rheumatic

disease One of the patients (no 17), who had suffered a classic rheumatic fever, had negative SCAT but increased ASF and slightly increased , globulin values

Our two patients with positive SCAT had been occupationally exposed to dust Case no 10 was a welder (see Case reports and figs 1a, 1b and 2) Haglind (10 11) described in 45 welders from the same shipyard roentgenologic lung changes similar to those seen in this patient before the onset of the current disease They had been exposed for 5 1 2 to 34 years Six patients with particularly protracted exposure periods were shown to have normal diffusion capacities The course was found to be entirely benign and in one who died of acute unspecific pneumonia, deposits of coal and iron rich pigments were observed at autopsy but no fibrosis (12) It is now generally accepted that welder s siderosis does not cause incapacitating lung injuries (1 13)

The other patient (no 11) had been exposed to silica Silicosis among workmen with this type of occupation (tunnellers) is however, rare in Sweden (1) The normal roentgen film eight years after the last exposure and 1 1/2 years before the relatively acute onset of the current disease rules out a direct relationship between the latter and exposure to dust The roentgen film and the gross picture had no similarity with that in silicosis and at microscopic examination only two small silicotic granulomas were found Neither the microscopic nor the roentgenologic pictures resemble the granuloma in pneumoconiosis in rheumatic patients described by Caplan (3 8)

A rheumatic factor could however, in these two cases be part of the pathogenetic mechanism Mattingly (19) reports cases in which a rheumatic joint disease was preceded with varying time intervals by a diffuse interstitial fibrosis in the lungs Such a sequel could obviously occur in the future in our cases with positive SCAT

Patho anatomically there is hardly any evidence in our series that IFL is rheumatic Neither fibrinoid degeneration or necrosis of collagen connective tissue nor rheumatic granuloma were observed Hyaline membranes were seen but they also occur in non rheumatic diseases

During recent years there has been increasing evidence to support the opinion that auto-immune mechanism are factors in the causation of IFL (15 16 22, 26) Mackay and Ritchie (17) recently reported two cases of diffuse fibrosing alveolitis (diffuse interstitial fibrosis of the lungs) with serological evidence of auto-immune reactions In 15 further cases with the same diagnosis there was a moderate incidence of positive reactions including positive rheumatoid factor in 24 % and positive auto immune complement fixation in 18 % The frequency of positive reactions greatly exceeded that among normal subjects

In our six cases — two with positive SCAT and one with marginal values — in which diffusion in gel analyses were carried out precipitating antibodies against lung antigen were not established This does not entirely exclude the possibility that specific lung antibodies occur The investigation must be regarded as a pilot study

Summary

Biopsy and or autopsy material was studied in 14 of the 19 patients with IFL who have been described in previous papers (2 18) A serologic and immunologic investigation was made on 14 cases

The following features were observed at biopsy and/or autopsy granulated lesions on the lung surfaces with small cysts in 11 cases, increased firmness in 12 major cyst formations in two, and signs of unspecified secondary infection in five cases

Microscopically, the fibrosis was more pronounced in the autopsy samples than in thoracotomy samples Newly formed connective tissue poor in cells, strongly proliferating alveolar epithelium inflammatory exudation and hyaline membranes were seen to a varying extent In two cases there was destruction of the epithelium in finer bronchi with intruding granulation tissues Honeycomb structures were observed in nine cases Intimal fibrosis of finer branches of the pulmonary arteries was recorded in two thirds of the cases

In five cases the lung lesions were more extensive than the lung roentgen films revealed in eight cases there was conformity and in one case appraisal could not be made Existence of honeycomb structures showed good conformity between radiologic and anatomic distributions

Grading of the disease by assessment of the findings at thoracotomy, palpation of the lung, evaluation of ventilatory resistance, microscopic examination and evaluation of the roentgen findings are proposed to be more reliable evidence for appraisal of prognosis and reversibility during steroid medication than only microscopic evaluation of the biopsy material

Positive SCAT was established in two of ten examined cases, both exposed to dust at work, but without evidence of significant occupational disease or clinical rheumatic disease These cases are discussed in some detail

The sera of six patients, two with positive SCAT, showed negative diffusion in gel analyses

Four selected cases, representing various courses and reactions to therapy, are presented in detail

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Infective Hepatitis and Toxic Jaundice in a Municipal Hospital During a Five-year Period

Incidence and prognosis

By

M Bjorneboe, O Iversen and Steen Olsen

To day, virus hepatitis is a relatively uncommon disease in Denmark (fig 1) In the 1940s the annual number of cases rose to about 18,000 During recent years the annual incidence has been only about 1 000 cases (Medical Reports of the Kingdom of Denmark, 1928—1965) In the 1940 the disease was relatively frequent in adults At that time many severe cases were seen in Danish hospitals with fatal results or subsequent development of cirrhosis (1, 2, 5) The prognosis was particularly unfavourable in females during and after the menopause Severe cases of hepatitis are now uncommon although it cannot be stated with certainty whether this is due to the low incidence of hepatitis or to the more benign course of the disease The present study was made with the object of elucidating the prognosis of hepatitis during the years 1960—1964 when the incidence of the disease was low Since it has become evident in recent years that several cases resembling hepatitis are provoked by various drugs, all cases of drug jaundice occurring during the same five year period have been registered and examined

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Material and methods

The basic material for the study comprises all patients with jaundice observed in the Department of Surgery A and the Department of Medicine B Bispebjerg Hospital from 1960 to 1964 There were 23 600 admissions to these departments during this period The material has not been selected but comprises all patients referred to these two departments from the Copenhagen Municipal Central Bureau for Allocation of Hospital Services Hence the patients represent a random selection of hospital patients in Copenhagen from 1960 to 1964 All record of patients with *jaundice* (i e serum bilirubin levels exceeding 1 5 mg per 100 ml) were reviewed and classified A major group of patients with mild jaundice

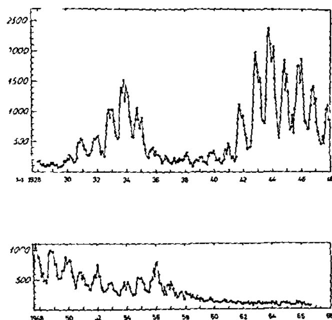


Fig 1 The incidence of hepatitis in Denmark from 1928 to 1966 (number of cases of acute hepatitis notified per month)

due to heart disease with liver congestion was not included in our material because analysis of their serum bilirubin values was not made systematically In the group of *gall stone diseases* the diagnosis was verified by operation or radiography in the group of *liver cirrhosis* it was confirmed patho anatomically in 90 % of the cases and in the *cancer* group by operation or autopsy The cases with *Gilbert Meulengracht s disease* include patients with chronic or recurrent jaundice normal liver biopsy normal radiographs of the biliary tract no signs of hemolytic anemia and normal levels of serum GO transaminase and alkaline phosphatase

The material per se comprises the groups of virus hepatitis and drug jaundice *Virus hepatitis* includes serum hepatitis and suspected infective hepatitis The diagnosis of hepatitis was made in patients who presented with an entity resembling hepatitis clinically and biochemically and had *not* been given hepatotoxic drugs during the period prior to the onset of the disease The diagnosis of *serum hepatitis* was made in patients who had received blood or serum transfusions during the preceding six months and the diagnosis of suspected infective hepatitis was made in those who had *not* received such transfusions The diagnosis of hepatitis was confirmed patho-anotomically whenever possible using the Menghini modification of Iversen and Roholm s liver biopsy technique The presence of gall stones was excluded by radiographic studies Biochemical studies included determination of serum bilirubin GO transaminase and alkaline phosphatase The transition from hepatitis to cirrhosis was studied whenever possible by repeated liver biopsies by the method first described by Krarup and Roholm (6) *Drug jaundice* was diagnosed when episodes of jaundice followed treatment with hepatotoxic drugs Gall stone diseases were excluded after radiographic studies Liver biopsy was performed whenever possible Biochemical studies include the same parameters as in cases of virus hepatitis

Results

Table I presents the classification of jaundice in the most important groups Gall stones, cirrhosis and cancer are the most frequent causes of jaundice The group headed 'miscellaneous includes four cases of hemolytic jaundice three cases of severe infective disease two heavily dehydrated patients one patient presenting an allergic reaction to penicillin and one with acute pancreatitis The five patients with Gilbert Meulengracht s disease were all young Liver biopsy was performed in four Further analysis of bilirubin in its conjugated and unconjugated forms was not made

In the group with *virus hepatitis* there were 11 cases of serum hepatitis and 24 cases of suspected infective hepatitis The cases of *serum hepatitis* developed 2—5 months after transfusions of blood or serum Table II shows the sex and age distribution of these patients Biopsies were performed in two cases, and autopsies were made on two patients who died during the acute phase the examination included microscopy of the liver Seven of the surviving nine patients were followed up 2—27 months after discharge, and the results are shown in table II One of the patients who died during the acute phase was a 64 year old male who had been operated on for cancer of the stomach and had received several transfusions in connexion with surgery He died two months after operation having had jaundice for 18 days Autopsy showed pronounced necrosis of the liver with no metastases The other patient was a 43 year old female, who had received transfusions because of severe anemia in connexion with menorrhagia Three months later she died having been jaundiced for four days Autopsy revealed acute atrophy of the liver In the remaining nine cases the jaundice persisted for periods of one to two and a half months In one patient a 65 year old female hepatic coma developed but disappeared after prednisone treatment It is believed that two patients developed liver cirrhosis because of the serum jaundice One

TABLE I Classification of cases of jaundice 1960—1964

	Cases	Per cent
Gall stones	305	47 7
Cirrhosis	141	22 0
Cancer	128	20 0
Virus hepatitis	35	5 5
Drug jaundice	15	2 3
Miscellaneous	11	1 7
Gilbert Meulengracht s disease	5	0 8
	640	100 0

TABLE II Serum hepatitis

No	Sex	Age	Follow up
1	♀	22	Healthy
2	♀	43	Died during acute phase
3	♀	58	Cirrhosis
4	♀	65	Healthy
5	♀	67	Healthy
6	♀	67	Cirrhosis Died from cancer of the stomach (see text)
7	♂	31	Healthy
8	♂	64	Died during acute phase
9	♂	65	—
10	♂	70	—
11	♂	74	Healthy

TABLE III Infective hepatitis

No	Sex	Age	Biopsy during acute phase	Follow up
1	♀	16	+	—
2	♀	24	+	Healthy
3	♀	42	—	Healthy
4	♀	49	+	—
5	♀	52	+	Cirrhosis
6	♀	55	+	Cirrhosis
7	♀	56	—	Healthy
8	♀	61	+	Cirrhosis
9	♀	62	+	Healthy
10	♀	66	+	Cirrhosis
11	♀	69	+	Healthy
12	♀	85	+	Died post operatively Cause pulmonary embolus (see text)
13	♂	18	+	Healthy
14	♂	21	—	Healthy
15	♂	22	—	Healthy
16	♂	37	—	Healthy
17	♂	37	+	—
18	♂	41	—	Healthy
19	♂	43	+	Healthy
20	♂	45	+	Cirrhosis
21	♂	46	—	Healthy
22	♂	49	—	Healthy
23	♂	62	—	Healthy
24	♂	74	—	Healthy

of them was a 67 year old female who had been operated on for cancer of the stomach and had been given transfusions in connexion with the operation Macroscopically the liver was found to be normal at operation Five months later she developed jaundice which was found to be of the hepatitis type by clinical and biochemical examinations The patient died two years later and autopsy revealed malignant metastases and pronounced portal cirrhosis The other patient was a 58 year old female who developed hepatitis verified by biopsy, four months after surgical evacuation of a cerebral haematoma, during which transfusions had been given Nine months later biochemical evidence of liver cirrhosis was found but the patient refused a second biopsy

Maximal values of serum bilirubin, GO transaminase and alkaline phosphatase during the acute attack of serum hepatitis did not distinguish cases resulting in death or cirrhosis from benign cases

The sex and age distribution of the 24 cases of *supposedly infective hepatitis* are given in table III Two probably contracted the disease during a stay abroad (Italy, Holland) The jaundice lasted from one week to two months One 85 year old woman died from pulmonary embolism two weeks after explorative laparotomy Twenty of the surviving patients were followed up from 1 to 69 months after discharge Four females and one male developed cirrhosis of the liver, verified by liver biopsies In one of these women (no 10) the pre icteric stage was remarkably long (two months) Three of the women with cirrhosis died in hepatic coma 1, 3 and 3 years respectively after the acute attack of hepatitis In two of these patients autopsy was performed and the diagnosis of liver cirrhosis was verified The fourth woman is still being followed as an out patient five years after the acute attack Her clinical and biochemical condition varies greatly, with good and bad periods

A 45 year old man (no 20) one of the 12 males with hepatitis, developed

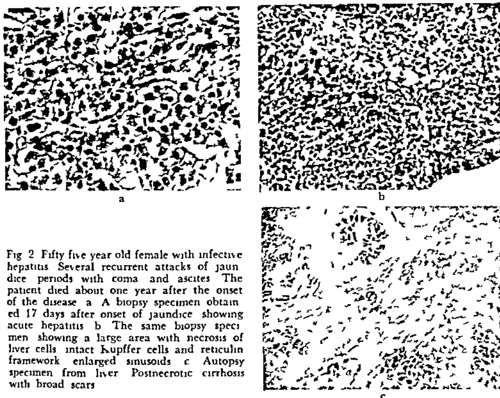
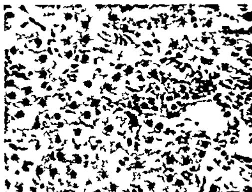


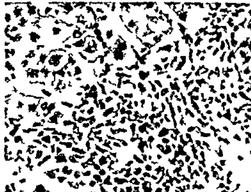
Fig 2 Fifty five year old female with infective hepatitis Several recurrent attacks of jaundice periods with coma and ascites The patient died about one year after the onset of the disease a A biopsy specimen obtained 17 days after onset of jaundice showing acute hepatitis b The same biopsy specimen showing a large area with necrosis of liver cells intact Kupffer cells and reticulin framework enlarged sinusoids c Autopsy specimen from liver Postnecrotic cirrhosis with broad scars

cirrhosis verified by repeated biopsies However, this patient may have had cirrhosis as early as his initial period of jaundice when he was first admitted to the hospital On two previous occasion he had been jaundiced and the biopsy specimen on the basis of which the diagnosis of hepatitis was made was very small, so that when reviewing the specimen it was not possible to decide whether it was a cirrhosis In another patient a 46 year old man (no 21) steatosis of the liver was found on repeated follow up studies including biopsy He was said to be an alcoholic The maximal values of serum bilirubin GO transaminase and alkaline phosphatase during the acute phase did not distinguish cases resulting in cirrhosis from benign cases

The histological picture observed in the biopsy specimens from virus hepatitis did not present any features which differed from the common findings (figs 2 and 3) It was marked by hepatocellular necroses with foci of leucocytes lymphocytic and monocytic infiltration of the parenchyma and the portal spaces as well as the presence of eosinophile cellular necroses The only case of serum hepatitis verified by biopsy did not differ histologically from the cases of infective hepatitis In ca es of hepatitis no distinct correlation was seen between the degree of the histological lesions in the biopsies and the subsequent course of disease In one case however in which cirrhosis developed extensive hepatic cell necroses were observed in particular



a



b



c

F g 3 Sixty six year old female w th nfec t e hepat t s a B opsy spec men obta ned about one month after the onset of jaund ce showing a ute hepat t s nflammatory cell inf ltrat on around the central ve n groups of hepa o-cellular nec oses b Three months later The b opsy spec men no shows pronounced act e c rrhos s w th rregular areas of prol ferat ng connect ve t ssue d ffuse del m tat on of the connect ve t ssue aga nst the l er cell trabeculae c B opsy sp c men obta ned 2 1/2 years after onset of the d sea e show ng stat onary c rrhosis n some areas w th slender collagenous connecti e t ssue stra ns wh ch segregate the lobules and n other areas w der collagen bands

centrolob ilarly fig 3) Three of the cirrhoses verified by histological examination were of the so called post necrotic type with broad scars The remaining cases could not be distinguished from simple portal cirrhosis without fatty changes



F g 4 Follow up study of 35 cases of hepat tis Cross hatched columns ind cate cases in wh ch cirrhos s developed Black columns show cases w th a fatal ou come during the acute phase One case w th development of c rrhos s s uncerta n (marked ()) One pat ent w th hepat t s d ed from pulmonary embol sm two weeks after operat on (marked ()) Abscissa age groups Ord nate number of cases

Finally it should be mentioned that three of the 35 patients with vir is hepatitis underwent surgery for diagnost c reasons As previously stated one of them an 85 year old female d ed from pulmonary embol sm two weeks after operation

The follo v up study of the 35 pati nts with virus hepatitis is summarized in f g 4

Drug jaund ce was diagnosed in 15 patients Li er biopsy was carried out in ten of these On the basis of biopsy findings and biochem cal observation six ca es were class fied as hepatitis (table

TABLE IV Drug jaundice of hepatitis type

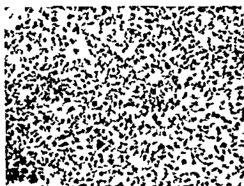
No	Sex	Age	Drug
1	♀	50	Chlorpromazine+Tanderil
2	♀	54	Halothane
3	♂	31	Trichlorethylene
4	♂	54	Paraflex
5	♀	66	Arcomonol+Plaquenil
6	♂	58	Butazolidin

TABLE V Drug jaundice of cholestatic type

No	Sex	Age	Drug
1	♂	85	Chlorpromazine
2	♀	65	Chlorpromazine
3	♂	68	Chlorpromazine
4	♂	69	Chlorpromazine
5	♀	34	Chlorpromazine+Trilafon
6	♂	50	Methyltestosterone
7	♀	55	Imipramin
8	♀	69	Tanderil
9	♀	49	Pyribenzamine (?)

IV) and nine as cholestatic jaundice (table V) Two cases have been published previously, one with jaundice after Halothane (12) and the other with jaundice after Imipramine (7) Age and sex distribution of the patients is given in the tables Maximum values of serum bilirubin GO transaminase and alkaline phosphatase were similar in severe cases (see later) and those who ran a more benign course

Five of the cases require further comment The case of Halothane jaundice occurred after the third and fourth anaesthesia employing this drug The case of Trichlorethylene jaundice was observed in a young man a narcomaniac, who inhaled this substance in large quantities The jaundice was extremely severe and anuria developed The patient recovered and was found to be well, from a clinical and biochemical standpoint at the follow up study three years later A 66 year old woman with severe chronic rheumatic arthritis developed hepatitis (confirmed by biopsy) after treatment with Arcomonol and


a


b

Fig 5 Sixty six year old female who developed jaundice associated with treatment with Arcomonol and Plaquenil a Biopsy specimen obtained four months after onset of jaundice when the serum bilirubin was 1 7 mg per 100 ml and serum GO transaminase and alkaline phosphatase were elevated The picture resembled hepatitis Nearly two years after onset of the disease a liver biopsy specimen showed cirrhosis (not illustrated) The patient died shortly afterwards Specimen of liver obtained at autopsy showed cirrhosis (b)



Fig 6 Fourty nine year old female with jaundice developing after treatment with Pyribenzamine Central lobular biliary obstruction with bile thromb (arrow) is seen as well as marked irregularity because of varying dimensions of the liver cells liver cell degeneration unicellular necroses and inflammatory cell infiltration

Plaquenil (fig 5) About two years later biopsy revealed a slight cirrhosis The patient died some months later from severe bed sores accompanied by staphylococcic infection and at autopsy the diagnosis of slight cirrhosis was verified A 69 year old woman developed cholestatic jaundice after treament with Tanderil At the same time a bleeding stomach ulcer occurred requiring operation which was followed by various complications resulting in death At most the hepatic disorder could have been a contributory cause of death A 48 year old woman developed cholestatic jaundice after treatment with Pyribenzamine a substance not previously known to cause liver damage (fig 6) The jaundice lasted for five months and explorative laparotomy and biopsy provided no explanation of the disease other than a possible drug jaundice Clinical and biochemical follow up studies seven months after discharge were normal Six of the remaining ten patients with drug jaundice were followed up from three months to four years after discharge Normal clinical and biochemical conditions were found in all these patients During the icteric phase two of the patients underwent surgery for diagnostic reasons (Impramin Pyribenzamine) Both endured surgery well As previously mentioned a third patient underwent operation for a bleeding stomach ulcer complicated by cholestatic jaundice after Tanderil treatment This operation was carried out on the vital indication of bleeding

Histological preparations were available from several cholestatic and hepatitic cases in the acute phase In the biopsy specimens from two patients with Chlorpromazine induced lesions and from three patients who had been given Methyltestosterone Impramin and Tanderil respectively histological signs of biliary obstruction were seen with the presence of bile thrombi mostly in the central lobular zone Furthermore in the patient who had received Tanderil moderate fatty changes were observed The biliary obstruction was accompanied by slight degrees of focal hepatocellular necrosis with associated slight leucocyte infiltration Conversely no severe diffuse or widespread liver cell necrosis or diffuse inflammatory infiltration was seen The picture did not resemble virus hepatitis In most cases the periportal space could be moderately oedematous with inflammatory cells In the patient who had received Pyribenzamine the biopsy specimen showed a picture resembling mixed cholestasis and hepatitis (fig 6) Two patients present

ing a picture of toxic drug hepatitis underwent biopsy soon after Halothane (12), and after Arcomonol and Plaquenil The biopsy specimens (fig 5) showed a diffuse inflammatory picture with widespread small hepatocellular necroses In the patient with Paraflex jaundice, only slight fibrosis of the liver was observed In this case the biopsy specimen was obtained six weeks after the onset of jaundice, at a time when the serum bilirubin value had returned to normal

Discussion

During the five year period of observation, virus hepatitis was an uncommon occurrence Out of 23 600 admissions only 35 cases were found For comparison it should be stated that the annual incidence in a Copenhagen municipal medical department, during the years 1941 to 1947 was 62 cases, against seven cases in the period covered here The incidence of hepatitis among icteric patients was about 40 % compared with 5.5 % in the present material (3) In this period from 1941 to 1947, hepatitis was a very frequent disease in Denmark However to-day the prognosis must still be considered serious, in particular in females Two patients (one of whom was female) died from acute liver atrophy, and seven (six of whom were female) developed cirrhosis which was fatal in three patients These are minimal figures for cases developing cirrhosis, as liver biopsy was not always performed at the follow up study Whether these patients actually had a virus hepatitis, cannot be stated with certainty The diagnosis was made on the basis of typical symptoms and by excluding all other causes of jaundice In practice these are the only criteria that can be employed The serious prognosis in our patients was presumably due to the fact that they were middle aged and elderly people, in whom hepatitis is known to carry a bad prognosis (1, 2, 4, 5, 9 10)

Relatively few cases of acute liver atrophy and post hepatitic cirrhosis were encountered during the five year period of observation, and this may be ascribed to the low incidence of hepatitis in the general population rather than an alteration of the seriousness of the disease Bjørneboe and Brøchner Mortensen in 1945 also advanced the opinion that the prognosis is identical at various times, when severe cases of hepatitis are related to the total number of cases of hepatitis in the population These authors compared cases of hepatitis with a serious outcome in a Copenhagen municipal medical department occurring in 1938 1939 and 1943/1944 They found that seven cases in 1938/1939 ran a severe course (with jaundice lasting for more than three months or death) against 32 cases in 1943/1944 These figures were correlated with the incidence of hepatitis in Copenhagen 40 per 100 000 in 1938 and 180 per 100 000 in 1943 It was, however emphasized that whereas three of the seven severe cases in 1938/1939 were women 24 of the 32 cases in 1943/1944 were women In 1960—1964 we have found nine cases of severe hepatitis (i e two per year) of whom seven were women The incidence of hepatitis in Copenhagen during these years was about 20 per 100 000 Our

figures are from another municipal medical department, with about 20 % fewer admissions per year, and include a few cases of hepatitis from a surgical department They are not strictly comparable with the figures from the paper quoted Nevertheless, they seem to reflect the same tendency, that the incidence of severe cases follows the incidence of hepatitis The preponderance of women among the severe cases noticed in 1943/1944 was also found in 1960—64

Drug jaundice must also be regarded as an uncommon disease over a period of five years, 15 cases were observed The responsible drugs are commonly recognized as potentially hepatotoxic substances, in particular Chlorpromazine and Butazolidin and its derivates (11) Pyribenzamine has previously not been reported to be hepatotoxic In four cases the disease was serious In one case liver damage was believed to be a contributory cause of death, in one case cirrhosis developed, in one case transient anuria occurred and in one case the jaundice lasted for five months

Five patients with virus hepatitis or drug jaundice were operated on for diagnostic reasons Four of them endured the operation well The fifth patient an 85 year old woman with a presumed infective hepatitis erroneously diagnosed as gall stones, died from pulmonary embolism two weeks after operation Surgery might have been avoided in three of the patients with virus hepatitis, if biopsy had been carried out previously The two patients with prolonged drug jaundice of the cholestatic type would probably not have avoided operation, irrespective of any diagnostic procedures being adopted, except possibly a percutaneous transhepatic cholangiography

Summary

All cases of jaundice encountered over a five year period (1960—1964) in one medical and one surgical department of a municipal hospital were reviewed and classified During this period 35 cases of virus hepatitis were found (six women and five men with serum jaundice, 12 women and 12 men with supposedly infective hepatitis) and six women and nine men with drug jaundice Most of the cases of hepatitis occurred in middle aged and elderly patients Forty one of these fifty cases were followed up Two of the patients with virus hepatitis died from acute liver atrophy (one male and one female) and seven (six of whom were females) developed cirrhosis, three of these patients died from this disease during the follow up period Four of the 15 cases of drug jaundice ran a severe course, and in two of them the liver disease was a contributory cause of death

It is concluded that hepatitis is always a serious disease in middle aged and elderly patients, especially in women The frequency of the serious consequences, death or cirrhosis, is rather an expression of changes in the incidence of hepatitis than of a change in the virulence of the disease Toxic jaundice is not a frequent disease but several cases run a severe course

Acknowledgement

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Cyclic Neutropenia

Report of a case treated with high doses of testosterone

By

Hakon Barkve

Cyclic neutropenia, also called cyclic agranulocytosis, periodic myelodystrophia or simply cyclic fever is a mysterious disease of unknown etiology A few reports suggest a familial occurrence (4, 9, 11) Although the syndrome has been known since 1910 only approximately 46 patients have been described among them eight from Scandinavia (1 7, 9 11)

At regular intervals, most often of about three weeks, the following symptoms recur neutropenia or agranulocytosis fever ulcerations of the oral mucosa often skin infections or inflammatory manifestations in other organs and sometimes also arthralgia, abdominal pain malaise and headache These symptoms usually last for 2—6 days and the patient is completely well between the attacks The typical clinical course and changes in the blood and bone marrow strongly suggest that the syndrome is a nosological entity There is no cure for this disease, but splenectomy and prednisone possibly had some effect in occasional cases (1, 4, 8 9 11)

The purpose of this article is to report a patient in whom the disease changed from a typically cyclic to a permanent and severe granulocytopenia Splenectomy and steroids had little or no effect Testosterone in large doses, however, had a remarkable clinical effect in spite of only a modest effect on the granulocytopenia

Case report

The patient is a male born in 1946 His parents and three siblings are healthy Except for some slight eczema in his mother s sister there are no allergic or other diseases in the family

In early infancy he had whooping cough with pneumonia mumps and chicken pox When four years old a tonsillectomy was done on account of fever and sore throat about every three weeks He was then well until October 1957 when he was hospitalized for skin infections

During the following years he suffered from infections at regular intervals of 2—3

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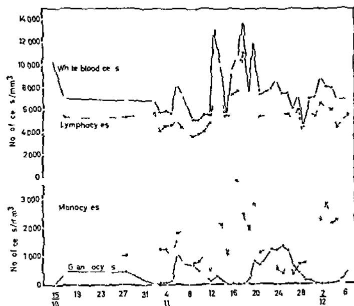


Fig 1 White blood cells and differential counts during three periods of agranulocytosis

weeks Before an outbreak he was moody tired and mentally instable Thereafter the fever rose to 39 C ulcerations appeared in the mouth and he often had diarrhea The fever usually lasted for a couple of days During the years from 1957 until December 1959 he was hospitalized several times in different hospitals Pyodermia alveolar pyorrhea axillary abscesses stomatitis pyuria and septicemia were the diagnoses He recovered with antibiotic treatment only to relapse at the expected time He was now bedridden for longer periods Neutropenia was observed twice but systematic differential counts were first carried out in the autumn of 1959 It then appeared that the granulocytes had a cyclic curve with agranulocytosis shortly before the peak of symptoms At the first day of agranulocytosis he was unwell had a slightly increased temperature and subsequently the oral mucosa became swollen with necrotic spots Always he began to feel better before the granulocytes reappeared in the peripheral blood

In October 1959 he was referred to this department On admission he had a large abscess in the face and the spleen was enlarged to 6 cm below the costal margin The clinical examination was otherwise negative

Peripheral blood

Hb was 65 % red cells 3 7 mill /mm³, and platelets 277 000 per mm³ Red cells and platelets appeared normal on smear White blood cells and differential counts were carried out every day during three cycles (fig 1) A total agranulocytosis developed every two weeks lasting each time for about four days As the granulocytes disappeared a compensatory monocytosis and lymphocytosis developed There was a slight eosinophilia and the neutrophilic granulocytes showed toxic granulation but abnormal cells were not found

Bone marrow

When the granulocytes were rapidly decreasing in the peripheral blood the bone marrow was completely dominated by promyelocytes and myelocytes only 5 % of the nucleated cells were band and segmented forms There was a slight eosinophilia Between attacks e g seven days after the last day of agranulocytosis the bone marrow

was normal except for slight eosinophilia and toxic granulation of the neutrophils

Bone marrow biopsies showed increased cellularity with small islands of lymphocytes among the myeloid and red cell precursors These were partly arranged around small vessels The bony lamellae were thick and there was a slight but definite fibrosis

Other tests

Coombs test was negative, and the serum did not contain Rh antibody or cold agglutinins Widal s test agglutination test for B abortus Paul Bunnel s test Dye test Meinicke s and Wassermann s test were all negative Antistreptolysin titer was normal while antistaphylolysin titer was increased to 16 LE cells were not found The ESR was moderately increased in periods with infection The serum protein level was about 8 g% and electrophoresis showed increased γ globulins of 2—3 g% Serum creatinin blood urea thymol turbidity bilirubin prothrombin time and transaminases (SGOT SGPT) were all normal The serum iron was 28—69 γ% The glucose tolerance test was normal A transfusion of 500 ml normal plasma during agranulocytosis had no effect on his white cells ECG and X rays of the bones and chest were normal In 1964 the excretion of 17 ketosteroids in urine was 1 1 mg/24 hrs and of 17 OH ketosteroids 1 9 mg/24 hrs Chromosome analysis showed a normal pattern Immun electrophoresis performed in 1964 and 1966 indicated slight elevation of γG γA and γM immunoglobulins

Treatment and course

Until November 1964 he was only treated with antibiotics The last years he had been slightly anemic and the platelet counts had shown a fall from 277 000 to 159 000 per mm^3 White blood cells were about 7 500 per mm^3 but the granulocytes never exceeded 6 % The spleen was enlarged Several attempts at bone marrow aspiration gave a dry tap but biopsies showed a very cellular marrow suggesting hypersplenism Splenectomy was therefore carried out in November 1964 The spleen weighed 650 g and three small accessory spleens were also removed Thereafter the Hb total white cells and platelets increased but the severe granulocytopenia and the clinical symptoms persisted He was treated with cortisone 50 mg/day for a short period without any effect

In June 1965 when he was 19 years old he had not yet come into puberty his height was 173 cm and weight 53 kg The skin infections and gingivitis persisted There were many small lymph nodes in the neck axillae and groins The liver was not enlarged From this time he received testosterone enanthate (Primoteston Depot Schering A G Berlin) 500 mg i m every week and this has completely changed his life He has been free of infections and has not used antibiotics at all The gingiva is still slightly swollen but the oral ulcerations have disappeared He has not developed acne The masculine sex stigmata have developed and he has grown up to a tall strong man feeling completely healthy but has as his only complaint a tendency to perspiration

Before treatment with testosterone the average number of granulocytes was 530 per mm^3 (eight counts during eight months) After treatment this number increased to a mean of 1 041 per mm^3 (twenty five counts during eight months) This increase is significant ($0\,05 > P > 0\,02$)

After 15 months the dose was reduced to 250 mg testosterone once a week and he still has no symptoms

Discussion

It is not possible to trace the exact beginning of the disease in our case but according to the history he had fever and sore throat every three weeks until he was tonsillectomized at 4 years of age The disease therefore probably started very early in life From 4 to

11 1/2 years he was subjectively healthy Thereafter the picture changed again and a strict periodicity continued until the patient was about 17 years old He then went into a chronic state with permanent granulocytopenia and tendency to infections

The underlying mechanism of the disease is not known Morley (6) has recently offered a new hypothesis He found a periodicity in the neutrophils of healthy individuals, with cycles of 14—23 days' duration He therefore suggested that this is a normally occurring biorhythm and that the disease is due to an exaggeration of this normal rhythm

Our patient had splenomegalia, which is a fairly common finding in cyclic neutropenia (9) The histologic studies of the spleen and liver showed in our patient a moderate fibrosis, and this has been a rather frequent finding in this disease The patient probably had slight hypersplenism, but the splenectomy did not influence the fundamental mechanism of the disease

Reimann (10) found that 50 mg of testosterone proprionate i m every week had no effect Brodsky et al (2), however, gave 600 mg testosterone enanthate every week to one patient, and observed marked subjective and objective improvement including improved granulocyte values Encouraged by this report we gave 500 mg i m once a week and obtained a marked improvement

The mechanism of the increased resistance to infections may be related to the increased levels of granulocytes even if this increase was quite modest

Males and females normally have similar granulocyte levels Usually there is no evidence of a hormonal imbalance in patients with cyclic neutropenia (8) Our patient was still not in puberty at the age of 19, and his levels of 17 keto- and 17-OH steroids were subnormal Several workers have indicated that testosterone increases the activity of both erythro and leucopoiesis (5,3) Our patient was slightly anemic (Hb about 12 g%) before treatment, but his hemoglobin rapidly increased to 15 g% He improved dramatically, and we believe that the change was due to treatment The testosterone doses used are very high, but we have not seen any marked side effects

Good therapeutic response to steroids given in cyclic doses has been reported (1), but our patient did not respond to cortisone

Until a more causal therapy can be offered, testosterone in high doses is recommended

Summary

A case of cyclic neutropenia is reported The patient, a young man of 20, has been ill from the first years of life He was subjectively healthy from 4 to 11 1/2 years of age At the age of about 17 the typical periodicity of the disease changed to a chronic neutropenia with a constant tendency to infections Antibiotics have been of some value Steroids and splenectomy had no definite effect After large doses of testosterone enanthate the granulocytes increased only slightly but the clinical picture changed totally Since the beginning of this treatment he has been practically free from the recurrent infections

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Bacterial Growth in the Dialysate Fluid and the Reaction of Peritoneum to Peritoneal Dialysis

By

JON GJESSING

The procedure of peritoneal dialysis requires the insertion of a catheter into the abdominal cavity, and thereby opens an avenue for bacterial growth Contamination is also possible from the continuous flow of dialysing fluid into and from the peritoneal cavity through the catheter and from the personnel who handle the dialysis bottles and inject drugs into them

The chief danger in peritoneal dialysis is therefore bacterial peritonitis The treatment of uremia and intoxication by this method, first described in 1922, was only accepted 25 years later when antibiotics were in general use It has since been demonstrated that with reasonable care in the preparation of the dialysis fluid and handling of equipment this hazard can be readily eliminated and the use of antibiotics in the dialysis fluid has further reduced the risk of infection

This study was made in order to obtain further information about the risk of bacterial infection during peritoneal dialysis

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Method

Specimens for bacterial culture were taken in every instance under the same sterile conditions from the final bottle of the dialysate fluid The sample was smeared on blood agar, hematin agar anaerobic blood plates and serum dextrose broth If there was no growth on the plates after 24 hrs the serum dextrose broth was smeared on a new blood agar and hematin agar The positive bacterial cultures were tested by the disc method for resistance to tetracycline and erythromycin

The dialysis fluid used had approximately the same electrolyte composition as serum except that no potassium was present

Composition of dialysis fluid sodium 140 4 mEq/l calcium 3 5 mEq/l magnesium 1 5 mEq/l chloride 101 0 mEq/l lactate 44 5 mEq/l glucose 15 g/l

In cases without pre existing hyperkalemia 4 mEq of potassium were added to every bottle of the dialysis fluid with 10 mg of Heparin and either 10 mg tetracycline

(Dumocyclin®) from Dumex or 10 mg Erythromycin® from Abbot In some cases no antibiotics were added to the fluid

Autopsies were performed on patients who died during or after treatment with peritoneal dialysis and samples of tissue from the peritoneal cavity were taken for PAD examination

Material

152 cases were studied (68 men and 84 women), their ages ranging from 5 to 90 years In 131 cases samples from the final dialysate fluid were examined for bacterial growth In 23 cases post mortem pathological and anatomical examinations were made on tissue from the peritoneum

In 11 cases of barbiturate intoxication an organic buffer (THAM) was added to the dialysis fluid to promote faster elimination of the barbiturate (4)

Results

Table I gives the results of the bacterial examination when different types of antibiotics used Bacteria were found in 56 of the 131 cultures (43 %) the most common being coli (36 %), staphyococcus aureus (34 %) staphylococcus albus (13 %) and enterococcus (13 %) Bacteria were found in 29 % of the cases when tetracycline was used, 42 % when erythromycin was used and in 68 % of the cases receiving no antibiotics

Bacterial resistance pattern

In the group with tetracycline added to the dialysis fluid, the growth of staphylococcus aureus was sensitive to erythromycin, but resistant to tetracycline, whereas the growth of coli was resistant to erythromycin but sensitive to tetracycline The same pattern was found in the group receiving erythromycin

In both these groups it was observed that there was a growth of bacteria which were sensitive to the type of antibiotic used

Table II gives the results of bacterial growth in samples from the last dialysate bath, the pathological reaction at the peritoneal cavity, the dialysis time, the antibiotic used and the length of survival after dialysis In the chronic nephritis group there was no reaction in the peritoneal cavity, in spite of the fact that bacteria were found in the dialysate

In the other groups it was found that more cases showed signs of peritoneal irritation, if bacteria were present in the

TABLE I Number of negative and positive cultures in the tetracycline no antibiotic and erythromycin groups

Type of antibiotic	No of cultures	Neg	Staph albus	Staph aureus	Coli	Enterococcus	Proteus	Alcaligenes	Fungus	Anaerobic	Difteroid	Alfa streptoc
Tetracycline	77	55	3	5	4	5	1	2	3	1	1	–
No antibiotic	28	9	2	8	8	1	1	3	–	–	–	–
Erythromycin	26	11	2	6	8	1	–	–	–	–	–	1
Total	131	75	7	19	20	7	2	5	3	1	1	1

TABLE II The relationship between the diagnosis culture of the last dialysate bath the reaction of peritoneum dialysing time and antibiotic used

Pat no	Diagnosis	Bacterial culture on the dialysate fluid	PAD	Duration of dialysis (hrs)	Dead after the dialysis started (days)	Antibiotic
1 a	Chronic nephritis	—	Neg	12	2	Tetracycline
32	Chronic nephritis	Neg	Neg	12	1	Tetracycline
71	Chronic nephritis	Pos	Neg	71	15	Tetracycline
68	Chronic nephritis	Neg	Neg	23 1/2	9	Tetracycline
74	Chronic nephritis	Pos	Neg	94	25	Tetracycline
72	Chronic nephritis	Pos	Neg	125 1/2	13	Tetracycline
145	Chronic nephritis	Neg	Neg	7 1/2	Same day	Erythromycin
18	Acute renal insuff	Pos	Pos	59	2	Tetracycline
19	Acute renal insuff	Neg	Neg	13	Same day	Tetracycline
30	Acute renal insuff	Pos	Pos	55	3	Tetracycline
35	Acute renal insuff	Neg	Pos	24	1	Tetracycline
37	Acute renal insuff	Neg	Pos	101	66	Tetracycline
46	Acute renal insuff	Pos	Neg	206	61	Tetracycline
137	Chronic pyelonephritis	—	Pos	337 1/2	21	Erythromycin
28	Amyloidosis	Pos	Neg	69 1/2	22	Tetracycline
41	Amyloidosis	Pos	Pos	134	10	Tetracycline
88	Acute pancreat	Pos	Neg	66	3	Tetracycline
37 a	Peritonitis	Pos	Pos	72 1/2	10	Tetracycline
61[1]	Intermittent dialysis	Pos	Pos	562 1/2	78	Tetracycline
73[2]	Intermittent dialysis	Pos	Pos	413	62	Tetracycline
109	Therapy resist oedema	Pos	Neg	75	7	No antibiotic
130	Therapy resist oedema	Pos	Neg	43	44	Erythromycin
143	Therapy resist oedema	Pos	Pos	23 1/2	13	Erythromycin

[1] Died after the last dialysis
[2] Died 6 days after the last dialysis

dialysate None of the cases with positive bacterial cultures or signs of peritoneal irritation showed signs of fulminant peritonitis

In four cases the dialysate fluid was positive at the beginning (peritonitis of the dialysis but negative at the end

Thirty three % of the patients with chronic nephritis receiving tetracycline gave positive results The dialysing time was about 40 % longer in these cases than in those which gave negative results, but this could not be verified in the barbiturate group

Eleven of the 18 cases of barbiturate intoxication received the organic buffer THAM in the dialysis fluid Three of these had positive cultures for staphylococcus coli and fungus respectively

During two periods the nose and throat of all members of the staff of the department were examined for bacteria

18 negative results and 8 positive were obtained in the first period, and 17 negative and 6 positive in the second period The bacteria found were staphylococcus aureus in 12 cases, enterococcus in one and proteus in one

Discussion

In this series the positive cultures found for the last outflow of dialysate did not imply peritonitis The dialysing procedure seemed to be effective against peritonitis, as shown by the four cases in whom peritonitis was present when the dialysis was started

The presence of bacteria in the outflow of dialysate, with no indication of the development of peritonitis has been verified by others (1, 3)

Schweinburg et al (7) have demonstrated a direct migration of bacteria across the intestine which presumably accounts for many of the positive cultures found Schwartz et al (6) reduced the incidence of bacterial infection by giving the patients prophylactic oral neomycin

In the present study tetracycline seemed to be more effective in reducing the bacterial growth than erythromycin Signs of toxic reaction, which were reported by Bulger et al (2), were not observed

The common finding of staphylococcus aureus in these patients seemed to have some relation to the high incidence of this organism in members of the staff

The number of positive cultures was higher in the chronic uremia group than in the intoxication groups In the chronic nephritis group the incidence of positive cultures was higher in those who were dialysed for a longer time This was not found in the intoxication groups, perhaps because these consisted of 'healthy' people

The addition of the organic buffer (THAM) (5) gave a more alkaline dialysate fluid with a pH of 8, making the fluid bacteriostatic except against enterococcus

Summary

Peritonitis is rarely observed in connection with peritoneal dialysis The risk of infection is higher during long term dialysis and in patients with chronic nephritis Tetracycline seems to be effective in reducing the incidence of bacterial growth in dialysate fluid

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Studies on Fatty Acid Metabolism in Diabetics During Exercise

VI Infusions of norepinephrine to male, non insulin treated, juvenile diabetics

By

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After a short period of muscular exercise male, *non insulin treated, juvenile* diabetics differed from control subjects by having a more pronounced rise in the plasma concentrations of free fatty acids (FFA) and glycerol (5, 6, 7, 9) This difference is apparently due to a more rapid and pronounced increase of the lipid mobilization from adipose tissue as there are no signs of an impaired elimination of glycerol from plasma in diabetics of this type (9)

The increase of lipid mobilization during exercise may be caused by higher sympathetic activity which may influence the adipose tissue either in humoral and/or neurogenic ways (12) Since a few years ago it is known that muscular exercise leads to higher plasma levels of norepinephrine (4, 25) Barany has shown that infusions of norepinephrine to diabetic patients caused a greater elevation of blood pressure than in control subjects (1) The present study was undertaken to find out whether a *higher sensitivity in adipose tissue to norepinephrine* could explain the difference found in lipid mobilization during exercise between juvenile non insulin treated diabetics and controls A preliminary report has been published earlier (5)

Material and methods

Five male, juvenile non insulin treated diabetics were selected for the study The patients (D1 D2 and D3) were all newly diagnosed and had never been treated with insulin. Patient D4 had been diagnosed as a mild diabetic four years previously and had received insulin for a short period However during the last three years good control was obtained by a regulated diet only Four weeks before the present examination his diabetic state got worse and he suffered from increasing thirst, polyuria and loss of weight. Patient D5 developed a mild diabetes about one year prior to the present examination and during that year was under good

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TABLE I Some clinical observations on the diabetic subjects

Case	Age (yrs)	Height/weight (cm/kg)	Plasma creatinine (mg/100 ml)	Duration of symptoms (weeks)	Complications
D1	33	170/65 3	—	?	0
D2	24	171/59 7	1 2	2	Suspect incipient diabetic retinopathy
D3	14	176/56 8	0 8	1	0
D4	28	180/57 8	1 0	Onset 1963 Under good control with diet only 1963—67 Actual symptoms for 4 weeks	0
D5	17	174/48 0	0 9	Onset 1966 Under good control with diet only 1966—67 Actual symptoms for 12 weeks	0

TABLE II Plasma FFA concentrations during the experiment (mEq/l)

	Time						
	Hours				Minutes		
Case	—2	—1 1/2	—1	—1/2	1	3	5
C1	0 54	0 37	0 38	0 49	0 58	0 68	0 81
C2	0 54	0 63	0 63	0 79	1 19	1 29	1 44
C3	0 38	0 39	0 44	0 52	0 53	0 58	0 67
C4	0 35	0 31	0 37	0 51	1 00	0 84	1 02
C5	0 57	0 44	0 67	0 85	1 02	1 09	1 21
Mean	0 48	0 43	0 50	0 63	0 86	0 90	1 03
S E M	0 05	0 05	0 06	0 08	0 13	0 13	0 14
D1	0 68	0 76	0 53	0 56	0 58	0 81	0 83
D2	1 18	0 85	0 74	1 22	1 36	1 51	1 54
D3	1 04	0 50	0 63	0 63	0 69	—	1 70
D4	0 66	0 42	0 44	0 51	0 65	0 80	0 88
D5	0 77	0 77	0 84	1 05	1 31	1 31	1 39
Mean	0 85	0 66	0 64	0 79	0 95	1 11	1 27
S E M	0 11	0 08	0 07	0 14	0 17	0 18	0 16

control with diet only. However 12 weeks before the examination he showed increasing thirst, developed polyuria and lost weight. After the present examination all patients have needed insulin therapy. Some clinical observations on the patients are summarized in table I. Patient D2 had a possible diabetic retinopathy whereas the others showed no signs of so called late diabetic complications.

Five apparently healthy subjects 22, 22, 24, 28 and 28 years of age were examined as controls. All had normal fasting glucose concentrations, no glucosuria and no family histories of diabetes.

The examination started in the morning, both the diabetics and the controls having fasted over night. One polythene catheter was inserted into the left brachial artery and another into the right cubital vein. Carbocain[P] (Mepivakain) without epinephrine was used for local anesthesia. The patients were then allowed to rest for 1 1/2 hours. After this period, norepinephrine was given as a constant intravenous infusion at a rate of 7.5 μg/min. The infusion continued for 10 min. The same dose was given to all the examined subjects. As the diabetic subjects weighed less than the controls (table V, $p < 0.05$) they were given a somewhat higher dose counted in μg/kg body weight. The average doses were 1.32 μg/kg for the diabetics and 1.16 μg/kg for the controls.

Blood samples were obtained through the arterial catheter for determinations of plasma FFA, plasma glycerol and blood glucose during the entire experiment at the time intervals indicated in tables II, III and IV. Blood samples for determination of acid-base balance were withdrawn before and during the infusion. ECG was registered during the entire experiment, intra arterial pressures were measured before and during the infusion. The whole experiment was performed at the Department of Clinical Physiology, University of Lund, Lund, Sweden.

Plasma FFA was titrated according to

8	11	13	15	18	25	40	55	70
—	0.83	0.81	0.86	0.75	0.70	0.52	0.39	0.39
1.32	1.56	1.84	2.01	1.64	1.40	0.82	0.53	0.74
0.79	0.95	1.02	1.03	1.05	0.89	0.58	0.40	0.39
1.07	1.62	1.30	1.43	1.58	1.25	0.64	0.58	0.81
1.35	1.49	1.64	1.70	1.54	1.15	0.78	0.61	1.15
1.13	1.29	1.32	1.41	1.31	1.08	0.67	0.50	0.70
0.13	0.17	0.19	0.21	0.18	0.13	0.06	0.05	0.14
1.04	1.16	1.29	1.30	1.36	1.22	0.95	0.80	0.57
1.98	2.24	2.48	0.95	0.94	1.08	1.58	2.14	2.43
1.76	1.46	1.28	0.96	0.86	0.91	0.86	1.09	0.93
1.04	1.11	1.50	1.02	1.16	0.81	0.56	0.58	0.68
1.56	1.78	1.98	2.12	2.22	1.97	1.26	0.92	1.17
1.48	1.55	1.71	1.27	1.31	1.20	1.04	1.11	1.16
0.19	0.21	0.23	0.22	0.24	0.21	0.17	0.27	0.33

TABLE III Plasma glycerol concentrations during the experiment (μ moles/l)

	Time						
	Hours				Minutes		
Case	—2	—1 1/2	—1	—1/2	1'	3	5
C1	32	31	37	37	49	63	80
C2	103	103	109	149	250	250	256
C3	43	31	51	46	47	66	93
C4	30	23	31	50	75	88	119
C5	47	34	71	70	88	114	149
Mean	51	44	60	70	102	116	139
S E M	13	15	14	20	38	35	31
D1	44	38	44	41	58	105	122
D2	53	41	36	72	132	174	222
D4	46	34	40	44	86	88	117
D5	56	54	69	84	128	135	167
Mean	50	42	47	60	101	126	157
S E M	3	4	7	11	18	19	24

TABLE IV Blood glucose concentrations during the experiment (mg/100 ml)

	Time						
	Hours				Minutes		
Case	—2	—1 1/2	—1	—1/2	1'	3	5
C1	80	78	77	79	80	76	81
C2	82	80	78	84	84	85	89
C3	77	77	76	78	74	72	77
C4	84	90	87	93	85	91	90
C5	93	86	84	86	93	79	88
Mean	83	82	80	84	83	81	85
S E M	3	3	2	3	3	3	3
D1	162	164	149	145	145	143	139
D2	199	198	195	192	190	189	192
D3	218	197	189	187	181	179	192
D4	209	214	204	206	210	205	209
D5	189	192	209	190	187	185	187
Mean	195	193	189	184	183	180	184
S E M	10	8	11	10	11	10	12

8	11	13	15	18	25	40	55	70
—	89	88	88	79	51	35	40	41
260	284	308	291	223	171	112	93	119
123	155	139	132	101	63	31	24	37
158	165	174	161	112	68	41	48	64
184	188	190	159	123	71	36	53	76
181	176	180	166	128	85	51	52	67
29	32	37	34	25	22	15	11	15
164	182	182	177	166	111	75	41	28
308	336	384	100	50	48	67	159	257
159	167	171	152	104	62	57	64	75
212	244	233	203	178	125	69	72	95
211	232	243	158	125	87	67	84	114
35	38	49	22	30	19	4	26	50

8	11	13	15	18	25	40	55	70
—	91	96	94	94	97	80	72	80
99	106	104	101	98	94	86	84	83
73	84	88	93	91	85	79	72	73
96	107	110	101	102	96	96	86	86
98	113	102	100	99	97	90	89	91
93	100	100	98	97	94	86	81	83
5	5	4	2	2	2	3	4	3
140	140	145	145	154	151	141	141	132
194	189	195	183	189	190	183	185	178
—	184	184	195	187	186	186	188	180
201	220	217	220	224	212	218	217	222
191	193	188	192	202	197	190	182	182
182	185	186	187	191	187	184	183	179
14	13	12	12	11	10	12	12	14

Dole (11) as modified by Trout et al (24) Blood glucose was determined according to Marks (20) as modified by Schersten (23), and plasma glycerol according to Laurell and Tibbling (19) The hemodynamic and acid base measurements were made by the routine methods of the Department of Clinical Physiology (8) The statistical evaluation was performed according to Wilcoxon s rank sum test (10)

Results

The hemodynamic data and the values of acid base balance are summarized in table V The norepinephrine infusion caused a lowering of the heart rate in both groups The mean decrease was somewhat greater in the diabetic group than in the controls, but the difference did not reach statistical significance The systolic diastolic and mean intra arterial pressures rose in both groups during the infusion The mean values of the diabetics and the controls were not significantly different either before or during the infusion, although the mean initial systolic pressure in the diabetic group tended to be lower than that of the control group This in turn caused a

TABLE V Age height weight hemodynamic data and base excess for the diabetics and the controls before and during the infusion of norepinephrine Mean±standard error of the mean No significant differences are found between the groups except in regard to weight ($p < 0.05$)

	Diabetics			Controls		
	n	Mean	S E M	n	Mean	S E M
Age yrs	5	23 2	3 5	5	24 8	1 4
Height cm	5	173 8	1 9	5	179 2	2 9
Weight kg	5	56 9	2 8	5	67 5	2 8
Heart rate beats min						
Rest	5	60 0	2 5	5	57 8	2 8
Infusion	5	49 8	2 9	5	54 0	2 3
Pressure in art brach						
Systolic						
Rest	5	110 4	4 4	5	122 8	6 7
Infusion	5	144 0	7 5	5	141 0	5 1
Diastolic						
Rest	5	64 4	1 7	5	68 2	1 6
Infusion	5	83 6	3 2	5	81 4	2 2
Mean						
Rest	5	83 0	3 1	5	88 8	3 1
Infusion	5	107 6	4 4	5	106 8	2 5
Base excess '						
Rest	4	—1 50	1 74	5	—1 10	0 80
Infusion	4	—1 62	0 72	4	—1 25	0 66

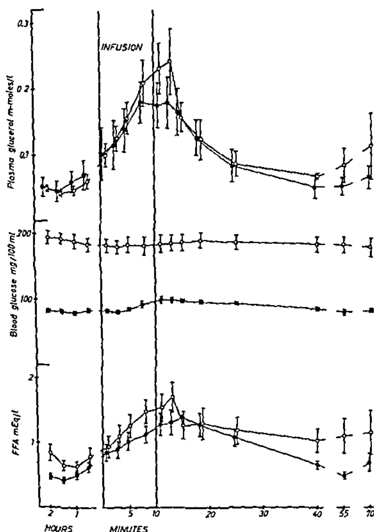


Fig 1 Plasma FFA blood glucose and plasma glycerol concentrations in the diabetic group (open circles) and the control group (closed circles) during the experiment Mean ± S E of the mean

tendency for the mean rise of blood pressures during infusion to be a little higher in the diabetics than in the controls but the differences were not significant

Thus the hemodynamic response was about the same in the diabetic group compared with the present control group and roughly with earlier findings using a similar technique in normal subjects (16) despite the fact that the diabetics had been given a slightly higher dose counted as μg norepinephrine kg body weight This finding may seem to differ from those of Barany (1) but in his diabetics the duration of the disease ranged from 6—22 years and thus his

results are not comparable with the present ones

The plasma FFA concentrations during the experiment for diabetics and controls are given in table II Two hours prior to the infusion the mean plasma FFA concentration of the diabetic group was higher than that of the control group ($p<0.05$) At all other times there were no significant differences between the diabetics and the controls The plasma glycerol concentrations are given in table III No significant differences were found between the diabetics and the controls, although a slight tendency to higher values was noted in the diabetics

In both the diabetics and the controls the infusion of norepinephrine caused a rise of the plasma concentrations of FFA and glycerol This is in agreement with earlier findings, when norepinephrine has been given to normal subjects and rises in plasma FFA (14, 18) and glycerol (3) have been noticed The rise has been explained as an increased lipid mobilization from adipose tissue (3) In the present investigation the same technique and dose of norepinephrine were used as described by Carlson and Oro (3), and the present results concerning the plasma concentration of FFA and glycerol in the controls correspond well with theirs

Thus the lipolytic response to the infused norepinephrine was not greater in the diabetic than in the control group, although a slightly higher dose, counted in μg/kg body weight, was given to the diabetics

The blood glucose concentrations are given in table IV During the entire experiment the mean blood glucose concentration of the diabetics was higher than that of the controls The controls exhibited a slight rise in the mean blood glucose concentrations during and for some minutes after the infusion Such a rise has been shown to occur after norepinephrine by other authors, e g Havel et al (16), and is thought to be due to glycogenolysis in the liver with an increased influx of glucose to the plasma No such elevation was apparent in the diabetic group, in which the blood glucose fell slowly during the observation period

Discussion

The cause of the increased mobilization of fat during exercise is not yet conclusively understood and has been under discussion by many authors during recent years (e g 2, 4, 12, 13, 15, 16, 17, 21, 22) However, as Hartog et al pointed out (12), the present evidence strongly suggests that the increase of lipid mobilization during exercise in normal subjects is primarily due to an increase of sympathetic activity As yet it is not quite clear whether the increased sympathetic activity is transmitted in humoral and/or in neurogenic ways Wirsen (26) proposed that the main sympathetic influence on white adipose tissue is transmitted humorally, but on the other hand Hartog et al (12) recently reported that the present evidence strongly suggests that rapid activation of hormone sensitive lipase in adipose tissue and consequent mobilization of fat during exercise result primarily from increased sympathetic nervous activity

If the lipid mobilization is caused by increased sympathetic activity both in the controls and in the earlier studied juvenile, newly diagnosed diabetics (5, 6 7, 9) the difference in lipid mobilization between these two groups may be explained in one of two main ways

1 Exercise causes a higher sympathetic tone in the diabetics
2 There is a higher sensitivity to nor epinephrine stimulation in the hormone sensitive lipase system in the adipose tissue of the diabetics than in that of the controls

As stated in the introduction, the present study was started to examine whether the second explanation was valid After infusion of norepinephrine no difference between the diabetics and the controls was found in regard to the lipolytic response as measured by the plasma concentrations of FFA and glycerol The present results suggest that no augmented sensitivity to norepinephrine stimulation exists in the hormone sensitive lipase system in juvenile, non insulin treated diabetics in comparison with control subjects Thus the higher mobilization of fat during exercise in diabetics of this type (5, 6 7, 9) is probably not due to the second explanation This may be true whether or not the sympathetic activity is transmitted by humoral or neurogenic means as nor epinephrine is the transmitter at the sympathetic nerve endings

Whether the higher lipid mobilization, known to occur in juvenile, newly diagnosed diabetics is caused by a higher sympathetic tone according to the first explanation above and/or some other agent or mechanism still remains unclear

Summary

Infusions of norepinephrine were given to five juvenile, non insulin treated diabetics and five control subjects In both groups the plasma FFA and the plasma glycerol concentrations rose by about the same magnitude and for the same duration which suggests that no increased sensitivity exists in the adipose tissue of the diabetics to norepinephrine stimulation The more pronounced lipid mobilization, earlier reported to occur in diabetics of this type during exercise is evidently not due to any increased sensitivity in the diabetic adipose tissue to norepinephrine

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Coronary Heart Disease and the Living Standard

By

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Increasing sugar consumption has recently been attracting interest as a possible factor in the etiology of coronary heart disease For instance, it has been stated that sugar has increased more than other dietary components in populations where a great increase in coronary heart disease has been observed (3 4, 5)

An increase in the consumption of sugar as well as in that of fat, are typical indicators of a rising standard of living and correlation between coronary heart disease and sugar consumption may in fact only mean a correlation between this disease and the level of the standard of living To study this point I compared the coronary mortality rate in 20 countries with the sugar and milk consumption number of radio and TV licences number of cars and the national income The figures are taken from official statistics for 1962 (table I) Table II shows the correlations between coronary mortality and the factors in question

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Table II shows a significant correlation between the male coronary mortality rate and many of the parameters of a high standard of living The correlation with the consumption of sugar is highly significant, and likewise that with the number of cars and the national income The correlation between the male coronary mortality rate and milk consumption and the number of radio and especially of TV licenses, is not equally close But the correlation is significant in general

In contrast with the situation for male coronary mortality no correlation was established between female coronary mortality and the parameters of a high standard of living Only with the number of cars was there correlation among women aged over 45

Discussion

In evaluation of the results it must be borne in mind that the figures are taken from official statistics of indeterminate

TABLE I Coronary mortality rate and parameters of living standard

	Mortality from myocardial infarction per 100 000 inhabitants					
	♂			♀		
	35—44	44—54	55—64	35—44	45—54	55—64
Australia	66 6	312 1	874 9	15 5	75 7	304 0
Austria	36 2	162 3	513 8	10 7	36 6	155 5
Belgium	37 4	141 0	402 2	8 5	33 1	115 3
Canada	70 3	304 1	814 5	12 0	58 7	272 3
Denmark	35 5	177 0	542 9	5 5	30 4	163 0
England	57 4	222 6	684 3	8 6	41 0	198 6
Finland	88 6	343 9	910 5	14 3	55 3	246 1
France	19 4	72 6	200 8	3 5	13 1	60 8
Greece	13 1	48 9	138 9	6 7	17 3	51 3
Israel	30 8	155 2	563 8	6 2	58 9	317 9
Italy	33 2	128 8	387 4	10 8	40 4	165 2
Japan	18 5	52 0	151 8	16 7	35 1	95 9
Netherlands	33 4	164 1	495 7	4 8	23 4	129 5
New Zealand	54 8	273 3	771 6	11 8	56 1	268 0
Norway	38 4	169 5	526 1	4 4	24 3	149 1
Sweden	19 9	127 2	494 4	4 4	24 8	162 1
Switzerland	33 8	130 2	445 5	8 3	35 9	167 3
U S A	90 6	358 8	923 7	18 5	81 4	313 1
Western Germany	41 5	170 0	500 2	15 1	47 9	173 2
Yugoslavia	7 5	72 0	324 5	5 7	49 1	249 7

[1] Estimated

TABLE II Correlations between coronary mortality rate and parameters of living standard

	Sugar consumption (kg/person)			Milk consumption (l/person)			No of radio licences (/1 000 inhab)		
	r	t	p	r	t	p	r	t	p
♂									
35—44	0 61	3 30	<0 01	0 52	2 57	<0 02	0 65	3 65	<0 01
45—54	0 69	4 03	<0 001	0 55	2 76	<0 02	0 62	3 31	<0 01
55—64	0 75	4 83	<0 001	0 60	3 14	<0 01	0 55	2 82	< 0 02
♀									
35—44									
35—44	0 04	0 15	>0 05	0 08	0 35	>0 05	0 44	2 07	>0 05
45—54	0 29	1 29	>0 05	0 09	0 39	>0 05	0 49	2 36	<0 05
55—64	0 43	2 00	>0 05	0 27	1 18	>0 05	0 39	1 78	>0 05

Sugar consumption (kg/person)	Milk consumption (l/person)	No of radio licences (/1 000 inhab)	No of TV licences (/1 000 inhab)	No of cars (/1 000 inhab)	National income (/person $)
[1]50	126	[1]206	[1]150	267	1340
38	178	292	56	83	780
30	108	333	111	115	1120
[1]46	124	495	237	294	1530
48	130	362	191	—	1290
50	143	306	239	147	1220
41	293	289	96	62	1020
[1]29	103	282	79	169	1180
[1]14	41	83	—	10	390
32	115	[1]217	—	100	710
[1]24	60	165	72	59	620
17	20	[1]199	[1]133	25	450
44	148	255	118	71	930
40	144	240	40	303	1400
41	225	270	54	108	1110
42	158	282	211	192	1690
45	163	122	53	123	1590
41	121	986	316	417	2450
31	89	327	137	120	1190
[1]18	77	115	5	7	—

No of radio and TV sets

No of TV licences (/1 000 inhab)			No of cars (/1 000 inhab)			National income (/person $)		
r	t	p	r	t	p	r	t	p —
0 56	2 88	<0 01	0 65	3 58	<0 01	0 63	3 48	<0 01
0 54	2 69	<0 02	0 71	4 29	<0 001	0 65	3 66	<0 01
0 50	2 48	<0 05	0 69	4 04	<0 001	0 65	3 59	<0 01
0 40	1 83	0 05	0 36	1 64	>0 05	0 28	1 22	>0 005
0 34	1 52	>0 05	0 57	2 95	<0 01	0 42	1 97	>0 05
0 25	1 09	>0 05	0 55	2 79	<0 02	0 41	1 92	>0 05

reliability and, in some cases, based on estimates only But even allowing for this, several of the correlations established for male coronary mortality are so highly significant that they cannot be dismissed

The results suggest that there is some relationship between the male coronary mortality rate and the standard of living However, they *do not* warrant the conclusion that a high standard of living in itself leads to increased coronary mortality The risk factor may equally well be one or several individual factors associated with a high standard of living

It is interesting to note that the same correlation does not seem to obtain between the female coronary mortality rate and the standard of living

Although there obviously is a correlation between the male coronary mortality rate and the level of the standard of living table I shows differences between individual countries which are inconsistent with the general rule The most interesting of these deviations is the 2—4 fold difference in the coronary mortality rate between Finland and Sweden a point which has been considered earlier (1) The difference is singular as these two countries are neighbours, have a long common history and similar ways of life Besides nationality, the main differences are the higher standard of living and more advanced industrialisation and urbanisation in Sweden The point perhaps receives further clarification from the definite difference in the coronary mortality rate observable in Finland between western and eastern parts of the country in the latter it is nearly double the rate in western Finland (2) Yet the standard of living is definitely higher in western than in eastern Finland Is it possible that the standard of living as such does not contain the risk of coronary mortality, but that the phase of transition to a high standard and urbanisation, with the attendant difficulties of adaptation, do carry this risk

It is obvious that well planned consistent international epidemiologic studies which assess the different risk factors and not just the few individual factors that have usually been considered so far, might shed additional light on the etiology of coronary heart disease

Summary

The author has compared coronary mortality rate in 20 countries with parameters of the standard of living such as the consumption of sugar and milk number of radio and TV licences and cars, and the national income

A highly significant correlation was established between the male coronary mortality rate and sugar consumption, number of cars and national income The correlation was smaller but nonetheless generally significant between coronary mortality and milk consumption and the number of radio and TV licences Between the female coronary mortality rate and the parameters of a high standard of living there was correlation only with the number of cars among women aged 45

In discussing the significance of the findings the point is emphasised that the coronary mortality rates of some

individual countries do not follow the general rule An interesting example of this deviation is the great discrepancy in the coronary mortality rate between Finland and Sweden

The importance of careful international epidemiologic studies for elucidating the etiology of coronary heart disease is stressed

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Book reviews

Advances in teratology Vol I Edited by D H M Woollam 297 pages 64 ill $12 50 Logos Press Ltd, London 1966

The volume comprises seven surveys which together present a very good orientation on present important lines of research within teratology The introductory chapter on Down s syndrome (mongolism) is by L S Penrose, one of the pioneers of modern genetics Penrose presents a survey of the different forms of chromosomal aberrations within no 21 which may be the reason for Down s syndrome, discusses the significance of maternal age, and points out that roughly one third of the cases are caused by other factors In the following chapter Mary F Lyon gives a detailed presentation of her inactive X theory Gordon C Brown deals with the results of epidemiological research hitherto concerning the possible teratogenic effect of virus infections, primarily rubeola and influenza Robert S Brent presents immunological points of view on teratology The teratogenic effect of the azo dyes is dealt with by F Beck and J B Lloyd, the effect of ionizing radiation by P Hicks and Constance J d Amato The final chapter, by R W Smithells, surveys the problem of drugs and human deformities First class illustrations, extensive references, author index and subject index add further to the value of the book Much care has obviously gone into the editing of the volume, and it is to be hoped that the publishers will be able to keep their promise of an annual issue of the series that has now started

BENGT ROBERTSON, *Stockholm*

Die Therapie der endokrinen Krankheiten By Miklo's Julesz and Kalman Kovacs 593 pages 31 ill $ 16 00 Akademiai Kiado, Budapest 1966

What does the reader expect from a textbook of endocrinology? I think this is a point often not considered by those undertaking the enormous task of writing such a book The specialist has enough textbooks covering every detail of the field New developments in diagnosis and therapy he learns from the large number of journals on endocrinology and metabolism Those practising endocrinology, not as specialists but as practising physicians or heads of hospital departments, need textbooks that give a brief account of the theoretical background and precise rules how to handle the different endocrine diseases and metabolic disorders

This book gives in each chapter an extensive report of what is known about the physiology of the gland The authors seem to have aimed at including as many references as possible, which makes the reading difficult and sometimes confusing The chapters on the therapeutic measures are better and more valuable While reading the book, I have often been puzzled by the opinions of the authors on the treatment of various disorders, but their experience seems to differ from mine in many respects

I cannot find that this textbook is of particular interest for Scandinavian physicians There are better ones in the English and German languages It may, however, serve a great purpose in the authors home and neighbouring countries

Rolf Luft, *Stockholm*

Acta Medica Scandinavica Vol 182, fasc 5, 1967

From the First Medical Service Sahlgren s Hospital University of Goteborg, Göteborg Sweden

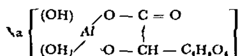
Antacids in the Treatment of Peptic Ulcer

By

G Dotevall and A Walan

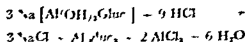
Peptic ulcers of the stomach and duodenum develop only in the presence of hydrochloric acid and pepsin, never in cases of true achlorhydria. On the other hand, hypersecretion of hydrochloric acid and pepsin is known to be a pre disposing factor in peptic ulcer disease The purpose of medical treatment of peptic ulcer is therefore to inhibit the secretion of hydrochloric acid with anticholinergic agents and to neutralize secreted acid with antacids When the gastric juice has a pH of more than 3 the peptic activity is much reduced which will give better opportunities for an ulcer to heal

The aim of this investigation was to study the effect on hydrogen ion concentration of two antacids in different doses and by various methods of administration These were tested by measuring the magnitude and duration of the change in gastric acidity when they were given alone and after anticholinergic treatment The first test substance was a suspension of aluminum hydroxide-magnesium carbonate gel (Almacid®, Draco AB, Lund, Sweden) Two different doses were used capable of neutralizing 18 mEq and 49 mEq HCl respectively in vitro The other antacid preparation (Ductal®, AB Astra, Soder talje Sweden) contained sodium gluconate-dihydroxyaluminate and had not previously been available in Sweden Its active principle has the following formula



Sodium gluconate-dihydroxyaluminate

This compound is an odorless substance with no disagreeable taste It dissolves at 25° C in water to give an 80 % (weight volume) solution and shows the following reaction with the HCl of gastric juice



$$3\,Na[Al(OH)_2Gluc] + 9\,HCl \rightarrow 3\,NaCl + Al\,Gluc_3 + 2\,AlCl_3 + 6\,H_2O$$

Submitted for publication March 20 1967

This substance was investigated in a biscuit form and as a liquid 40 % solution of sodium gluconate-dihydroxyaluminate with sucrose Ten ml of this solution has a neutralizing capacity of 18 mEq HCl in vitro

The composition of the antacid biscuit is as follows

Sodium gluconate dihydroxy-aluminate	0 69 g
Fat	0 83 g
Protein	0 26 g
Carbohydrates	2 39 g
Inorganic salts and water	0 12 g

One biscuit is equivalent to about 18 calories and neutralizes 6 0 mEq HCl in vitro

Thirty seven patients with radiologically verified duodenal ulcers were studied Each patient was investigated two or three times with drugs or water The order of testing was randomized and an approximately equal number of patients received either the drug or water first

Methods

The tests were carried out in the morning after 12 hours fasting and abstention from smoking With the patient seated comfortably in a semi recumbent position a gastric tube was introduced via the nose Two ml portions of gastric juice were aspirated at 10-min intervals At each fresh aspiration the tube was emptied by injection of air the first portion reinjected and a test portion drawn after repeat aspiration A radiometer pH meter was used for pH determination On completion of the initial pH determinations the relevant test substance was administered per os together with sufficient tap water to make a total fluid volume of 100 ml At 10-min. intervals thereafter the pH was measured again

When at least two determinations gave pH readings equal to or below the initial level another test series was begun. No patient was subjected to more than two different studies in one day

In 12 cases the effect was studied of antacid alone and when given two hours after administration of an anticholinergic agent (l hyoscyamine in sustained release tablets Egazil Duretter®, AB Hassle Gote borg, Sweden) in the optimal effective dose (10) Only one test series was undertaken on a single day in these patients

Another 13 patients were investigated by observing the pH changes that followed the intake of 100 ml water

In eight patients studies were made with sodium gluconate dihydroxyaluminate in biscuit form and in fluid form after ingestion of 500 ml whole milk two hours before beginning of the investigation

To have better opportunities to compare the effect of an antacid at different hydrogen ion concentration the pH values were converted to mEq/l using the tables given by Moore and Scarlata (6), where the activity coefficients at various pH levels had been taken into account As pepsin activity is markedly reduced above pH 3 the ability of an antacid to raise pH above this level was calculated and the time taken is given in minutes

Results

I *Effect of aluminium hydroxide magnesium carbonate gel*

The antacid effects of aluminium hydroxide magnesium carbonate gel with an in vitro neutralizing capacity of 18 and 49 mEq HCl (3 7 and 10 ml) were studied in 10 patients The initial hydrogen ion concentration was 16 0 and 16 8 mEq/l respectively The lowest recorded values, which were noted in the 10 min samples for both groups, were 1 74 and 0 96 mEq/l The 60 min samples contained 15 2 and 14 6 mEq/l As seen in fig 1, the values for the whole

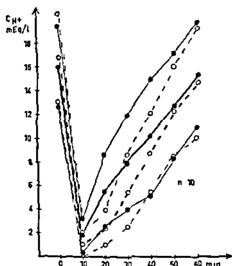


Fig 1 Effect on hydrogen ion concentration of different doses of aluminium hydroxide magnesium carbonate gel suspension ● = 3 7 ml with an in vitro neutralizing capacity of 18 mEq HCl ○ = 10 ml with an in vitro neutralizing capacity of 49 mEq HCl Mean ± S E

period in both groups were similar and no statistically significant differences were observed The mean duration of the elevation of pH values above 3 was 33 9 min when 3 7 ml aluminium hydroxide magnesium carbonate was given and 37 8 min when 10 ml was given (table I) After a mean duration of 48 9 and 52 2 min respectively the pH returned to its initial level There was no statistically significant difference between these groups Thus an almost threefold increase in antacid dose did not give a significantly better effect

II *Effect of aluminium hydroxide magnesium carbonate versus sodium gluconate dihydroxyaluminate*

Paired randomized studies were performed in nine patients to compare aluminium hydroxide-magnesium carbonate gel and sodium gluconate dihydroxyaluminate biscuits The patients were given 3 7 ml of the former preparation and three biscuits with water to a total volume of 100 ml Both these doses had neutralizing capacities of 18 mEq HCl in vitro The results are given in fig 2 and table II No statistically significant differences were found The mean initial hydrogen ion concentration was 20 0 mEq/l before the administration of the sodium gluconate dihydroxyaluminate and 16 0 mEq/l before aluminium hydroxide magnesium carbonate gel was given

As seen in table II the mean duration of pH ⩾ 3 and the time taken to return to the initial pH levels were not statistically different with the two preparations

TABLE I Effect on pH in gastric juice of aluminium hydroxide magnesium carbonate in gel suspension in two different doses with in vitro neutralizing capacity of 18 mEq (3 7 ml) and 49 mEq (10 ml) HCl respectively Mean ± S E

Dose (ml)	No of pats	Time for pH > 3 (min)	Time until return to initial pH (min)
3 7	10	33 9 ± 8 4	48 9 ± 6 7
10		37 8 ± 9 4	52 2 ± 9 1

The differences are not statistically significant (p > 0 05)

TABLE II Effect on pH in gastric juice of aluminium hydroxide magnesium carbonate gel suspension and of sodium gluconate dihydroxyaluminate in biscuits with in vitro neutralizing capacity of 18 mEq HCl Mean ± S E

Substance	No of pats	Time of pH > 3 (min)	Time until return to initial pH (min)
Aluminium hydroxide magnesium carbonate	9	33.9 ± 8.4	48.9 ± 6.7
Sodium gluconate dihydroxyaluminate	9	27.8 ± 8.9	38.3 ± 5.3

The differences are not statistically significant ($p > 0.05$)

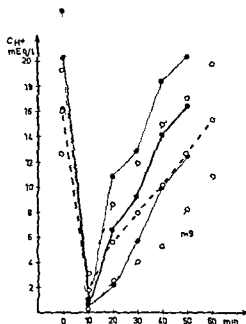


Fig 2 Effect on gastric hydrogen ion concentration of two different antacid preparations with equal in vitro neutralizing capacity (18 mEq) o = aluminium hydroxide magnesium carbonate gel suspension. ● = sodium gluconate-dihydroxyaluminate in biscuits Mean ± S E

III *Effect of sodium gluconate dihydroxyaluminate in biscuits and in solution*

To study the effect of different forms of an antacid sodium gluconate dihydroxy aluminate was given to eight patients in biscuits and in a solution The investigations were done in a random order Two hours before the antacid was administered the patients were given 500 ml whole milk The results are given in table III

The biscuit form of sodium gluconate-dihydroxyaluminate gave a mean duration of pH≥3 of 73 min while the corresponding figure with the solution was 40 min The difference (33 min) was statistically significant The mean time taken to return to the initial pH was 86 min with the biscuits and 44 min. with the solution This difference was also statistically significant The biscuit form of antacid raised the pH to higher values than the solution (pH 6.6 and 4.9 respectively) As can be seen there was a statistically significant prolongation of the antacid effect when it was given in biscuits

TABLE III Effect of sodium gluconate-dihydroxyaluminate in biscuits and in solution after previous ingestion of 500 ml whole milk Mean ± S E

No of pats	Time for pH ≥ 3 (min)		Time until return to initial pH (min)		Maximal pH	
	Solution	Biscuits	Solution	Biscuits	Solution	Biscuits
8	40	73	44	86	4 9	6 6
Difference	33		41		1 6	
S E	10 3		7 7		3 5	
p	<0 05		<0 05		<0 05	

IV *Effect of water*

In all investigations water was given to a total volume of 100 ml To investigate the effect of water alone on hydrogen ion concentration, 13 patients were given 100 ml The results are given in fig 3 The mean hydrogen ion concentration was not reduced below 10 mEq H/l In no case was the pH raised by more than 0 5 units and never above pH 3 The mean time taken to return to the initial hydrogen ion concentration was 31 9 min This is less than the respective values for the tests in which antacid preparations had been used

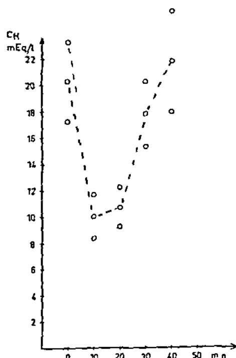


Fig 3 Effect on gastric hydrogen ion concentration of ingestion of 100 ml water Mean ± S E

V *Effect of anticholinergic treatment on duration of antacid action*

Paired studies were done on 12 patients who were treated with optimal effective doses of 1 hyoscyamine in sustained release form (1, 2) The anticholinergic drug was given two hours before the test On another day the study was repeated without anticholinergics Equal doses of the antacid were given on both occasions Without anticholinergic treatment the duration of pH ≥ 3 was 28 9± 6 1 min and with anticholinergic treatment 71 1 ± 12 0 min (table IV) The difference was statistically significant During treatment with the optimal

TABLE IV Effect on pH in gastric juice of antacids before and during treatment with optimal effective doses of an anticholinergic agent

	No of pats	Antacid alone	Antacid + anti cholinenergic agent	
Time for pH > 3 (min)	12	28.9 ± 6.1	71.1 ± 12.0	$p < 0.01$
Time until return to initial pH (min)		50.0 ± 4.8	75.6 ± 10.4	$p < 0.05$

The anticholinergic drug (l hyoscyamine) had been given two hours before beginning of the test

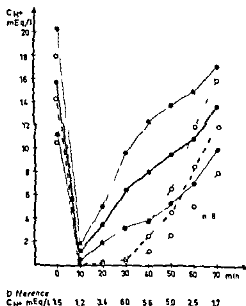


Fig 4 Effect on hydrogen ion concentration of an antacid with an in vitro neutralizing capacity of 18 mEq HCl before (●) and during (○) treatment with optimal effective doses of an anticholinergic agent Mean ± S E

effective dose of l hyoscyamine the initial pH varied between 1.8—4.75 with a mean of 2.60 Without anticholinergic treatment the initial level varied between 1.5—2.7 with a mean of 2.05 Anticholinergic treatment thus gives a higher basal pH and has a marked capacity to prolong the effect of an antacid preparation

Fig 4 shows the results obtained in eight patients who were given antacid, with an in vitro neutralizing capacity of 18 mEq, before and during treatment with optimal effective doses of l hyoscyamine Mean values for hydrogen ion concentration during treatment with l hyoscyamine were lower for every period but the differences were not statistically significant

Discussion

The effectiveness of an antacid is usually indicated in terms of its HCl neutralizing capacity in vitro However, in vitro studies are of limited clinical value It is necessary to consider also the quantitative secretion of gastric juice as well as the grade of emptying of the stomach

During recent years in vivo studies have been used increasingly in evaluating drugs used in treatment of peptic ulcer especially antacids Intragastric pH has been determined chiefly with special glass electrodes that permit in situ recording of pH in the stomach Results of

such measurements have been reported by Rovelstad and Maher (8), Tomenius and Williams (11) and Marcussen (5) among others

Following the administration of aluminium hydroxide magnesium hydroxide gels (15 ml) gastric pH, determined by such an in vivo technique, was above 3 5 for a period of 0 3 min (8) Brief effects on gastric pH were noted after ingestion of milk, cream or food With this antacid the duration of reduced gastric acidity averaged 8 min These studies used unguarded electrodes which *permit direct contact with the* gastric mucosa, and Rovelstad (9) considered the results to be unreliable because a guard was not used

Tomenius and Williams (11) on the other hand reported much longer effects of various antacids on the gastric pH They modified the method for intragastric determination by insulating the glass electrode and thus preventing direct contact with the stomach wall Direct contact could result in misleadingly high pH readings if mucus acted on the electrode, while the values would be too low if the hydrogen ion reached the electrode before it was neutralized by the administered antacid Tomenius and Williams also noted marked fluctuations in gastric pH with different positions of the electrode and different positions of the patient Uncertainties with the reference electrode can give an error as large as 0 7 pH units

To avoid these uncertainties with the in situ recording glass electrode, we decided to use the aspiration method in which small portions of gastric contents are taken for pH determination. Intake of liquid with the antacid ensured a closer approximation to physiologic conditions We attached particular importance to emptying the tube and mixing the gastric contents Small amounts (2 ml) were aspirated for pH determination These precautions reduced the sources of error that have been discussed by other authors (7)

The antacid effect of both aluminium hydroxide magnesium carbonate gel and sodium gluconate dihydroxyaluminate biscuits lasted for only about 40—50 min when given fasting The duration *of the effect on pH was approximately* equal for the two preparations An almost threefold increase in the dose of one antacid lengthened the duration of antacid action by only about 5 min (table I and fig 1) It is thus imperative to administer an antacid at frequent intervals if other measures that give a retardation of gastric emptying have not been taken The results obtained when antacid was given two hours after ingestion of whole milk indicate that this gives a marked prolongation of antacid duration These observations are in agreement with the results reported by Fordtran and Collyns (3) who gave antacid one hour after a meal composed of a sirloin steak In these conditions 4 g of calcium carbonate gave a depression of hydrogen ion concentration for at least three hours

The pronounced difference in antacid duration between antacid in biscuits and in solution is somewhat surprising The marked prolongation of action when given in biscuits could partly be the result of a retardation of gastric emptying induced by the fat (2 5 g) in the

biscuits It is however, unlikely that this can explain the marked difference that was recorded Gianturco (4) has demonstrated that radioopaque liquids do not mix with food but can pass beside it and leave the stomach within a few minutes Liquid solution of an antacid may leave the stomach faster than solid antacids when foods also occupy the stomach

Fordtran and Collyns (3) also investigated the effect of an anticholinergic given in optimal effective doses on the duration of the antacid In their experiments antacid was given one hour after a meal Mean values for hydrogen ion concentration were lower when an anticholinergic was given but the differences were not statistically significant In our study however a marked statistically significant prolongation of the antacid effect was noted when given during anticholinergic treatment This is c d by the depression of gastric secretio ch we previously found can be give l hyoscyamine in sustained relea f 2 This will give a lower hydr n cncentration which we also c n this study and it will give olume on which the antac d

Summary

1 Equiva oses of aluminium hydroxide m im carbonate gel suspension sodium gluconate dihydroxyaluminate in biscuits were studied with respect to the duration of antacid action No statistically significant differences were noted Both caused a reduction of hydrogen ion concentration for about 40—50 min and an elevation of pH above 3 for about 30 min

2 When aluminium hydroxide magnesium carbonate gel suspension was given in different doses with in vitro neutralizing capacity of 18 and 49 mEq HCl no statistically significant difference in duration of antacid action could be noted

3 After ingestion of 500 ml whole milk there was a marked prolongation of antacid action of sodium gluconate dihydroxyaluminate in biscuits when compared with equivalent doses of the same antacid in liquid form

4 Treatment with optimal effective doses of an anticholinergic agent (l hyoscyamine in sustained release form) gave a marked prolongation of antacid action When antacid was given alone the pH was elevated above 3 for about 30 min while the same dose of antacid given during treatment with anticholinergics resulted in an elevation of pH above 3 for about 70 min Anticholinergic treatment also resulted in a lower initial hydrogen ion concentration

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Correlation Between the Hyperkalaemic ECG-changes and the Potassium, Magnesium and Calcium Levels in the Serum in Renal Failure

By

Johan von Knorring and Börje Kuhlbäck

In studies on isolated frog hearts Kraus and Zondek (7), in 1938, noted electrocardiographic changes associated with elevated extracellular potassium levels. Later, Winkler et al (15) observed a close correlation in dogs between the serum potassium level and the electrocardiographic changes on intravenous administration of KCl. Pointed tent-shaped T waves in the unipolar precordial leads, in particular, are well known signs of moderate hyperkalaemia. Text books on electrocardiography also describe decreased P amplitude at higher serum potassium levels, prolonged P—Q interval, A—V block and lengthening of the QRS complex with prolongation of the Q—T duration.

The good correlation between progressing hyperkalaemia and increasing electrocardiographic changes observed in experimental studies does not, however, hold equally well for human subjects. The present study is concerned with the electrocardiographic changes associated with rising potassium levels in 40 patients with serum potassium values over 6.5 mEq/l. Particular attention was paid to the lack of correlation sometimes observed between severe hyperkalaemia and electrocardiographic signs of this condition. The effect of hypocalcaemia and hypermagnesaemia on the ECG and the possible correlation between these states and the electrocardiographic changes due to hyperkalaemia were also studied.

Material

Between Sept 1961 and Feb 1966 50 patients were dialyzed for hyperkalaemia ($K > 6.5$ mEq/l) and uraemia at the Renal Ward, Fourth Department of Medicine (Maria Hospital). The total number of dialyses was 65. Apart from ECG the routine examinations preceding dialysis included serum potassium, sodium, calcium and magnesium. In ten cases however the ECG was taken after the dialysis had been started

Submitted for publication March 29 1967

or it had been taken just previously at the remitting hospital These patients were omitted from the series since simultaneously determined ECGs and serum potassium values were lacking Of the remaining 40 patients 24 had acute tubular necrosis of different aetiology four had post renal anuria of short duration and three had acute glomerulonephritis as their basic disease In the remaining seven cases either chronic pyelo or glomerulonephritis in acute or terminal stage was the cause of the hyperkalaemia It was regarded as desirable *to exclude severe clinically observable pe* rimyocarditis and this condition did not seem to be present in any of the acute cases Pericardial friction rubs were likewise absent in the chronically ill patients, but some of them had roentgenologically demonstrable cardiac hypertrophy and/or hypertension

Methods

The ECGs were run at a speed of 50 mm/sec and calibrated so that 1 mV corresponded to 1 cm Apart from the standard leads the unipolar leads from the extremities and the unipolar precordial leads were *taken*

The following electrocardiographic changes were regarded as indicative of hyperkalaemia or of hyperkalaemia in combination with hypermagnesaemia and hypocalcaemia

1) Narrow tent shaped T waves The amplitude of the T wave in lead V_3 was measured

2) Prolongation of the P—Q interval The P—Q interval was corrected by pulse frequency as suggested by Ashman and Hull (2)

3) Reduced P amplitude widening and or disappearance of the P wave leading to A—V block with idioventricular rhythm or supraventricular tachycardia

4) Broadening of the QRS complex over 0 11 sec.

5) *Prolonged Q—T_c duration* QT_c = the Q—T duration corrected by pulse frequency (5) The maximal Q—T_c duration is 0 43 sec for women and 0 42 sec for men Prolonged Q—T_c duration alone without any other signs of hyperkalaemia was not regarded as indicative of this condition but as signifying hypocalcaemia and/or hypermagnesaemia

6) S—T *depression of the so called* sludging type with a deep S deflection as a rule associated with QRS broadening

Serum potassium and sodium were determined by flame photometry Serum calcium was determined as suggested by Saris (11) *and magnesium by Andreasen s titan yellow* method (1) All these electrolyte determinations were performed at the same time as the electrocardiography The following values were regarded as borderline 6 5 mEq/l for hyperkalaemia 2 2 mEq/l for hypermagnesaemia and 9 mg/100 ml for hypocalcaemia

Results

In table I the series has been classified according to serum potassium level in order to assess the frequency of normal *and hyperkalaemic ECGs, respectively*, in the presence of potassium values over 6 5 mEq/l Of 40 patients with values over 6 5 mEq/l, 27 exhibited electrocardiographic changes consistent with hyperkalaemia It is also seen that the number of normal ECGs clearly decreased with a rise in serum potassium Levels over 8 mEq/l were invariably associated with obvious hyperkalaemic electrocardiographic changes On the other hand 9 15 patients with potassium values between 6 5 and 7 mEq/l had normal ECGs or ECGs with no signs of electrolyte disturbance In table II the 13 patients without electrocardiographic changes suggestive of hyperkalaemia are compared with those whose ECGs exhibited hyperkalaemic patterns, in both

TABLE I Number of ECGs with and without changes consistent with hyperkalaemia in 40 patients with serum potassium levels above 6 5 mEq/l

	Serum potassium (mEq/l)				Total
	6 5—7 0	7 1—7 5	7 6—8 0	>8 0	
No of pats with ECG patterns suggesting hyperkalaemia	6	6	7	8	27
No of pats with normal ECG patterns or with non specific changes	9	3	1	—	13
Total	15	9	8	8	40

TABLE II The distribution of normal ECGs and ECGs suggesting hyperkalaemia in acute or chronic renal failure with serum potassium levels above 6 5 mEq/l

	Acute	Chronic	Total
No of pats with ECG patterns suggesting hyperkalaemia	20	7	27
No of pats with normal ECG patterns or with non specific changes	11	2	13
Total	31	9	40

TABLE III Electrocardiographic findings consistent with hyperkalaemia at different potassium levels above 6 5 mEq/l in the patients presented in tables I and II

	Serum potassium (mEq/l) 6 5—7 0 (n = 15)	7 1—7 5 (n = 9)	7 6—8 0 (n = 8)	>8 0 (n = 8)
Tall slender T waves	5	5	6	7
Lengthening of the P—R interval	—	2	5	3
Disappearance of P waves	1	1	1	2
Increased QRS interval	—	—	1	4
Prolonged Q—T_c interval	4	1	3	4
S—T depression	1	1	2	3

groups the material is classified according to renal disease The distribution of acute and chronic renal insufficiency is more or less the same in the two groups On the other hand the group without hyperkalaemic changes contained a greater number of patients with hypertrophic hearts or hypotonia with a systolic pressure under 100 mm Hg This point will be discussed later

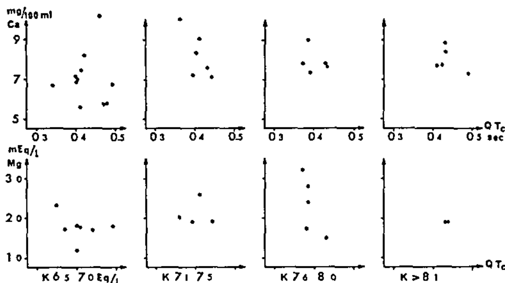


Fig 1 The influence of the serum calcium and magnesium levels on the Q T_c interval at different levels of hyperkalaemia above 6 5 mEq/l in the 40 patients of this series

The frequency of the various electrocardiographic signs of hyperkalaemia in the groups with various serum potassium levels is shown in table III

The frequency of typical, tent shaped T waves and the mean amplitude of the T waves showed an increase corresponding to the rise in serum potassium A prolongation of QRS occurred only at potassium levels over 7 6 mEq/l The other electrocardiographic changes also increased in frequency with rising serum potassium levels ST depression of a particular rising type from a deep sludging S deflection occurred in some cases often in combination with prolongation of the QRS interval as in right bundle branch block A decrease in the amplitude of the P wave, or widening of the latter, occurred at all potassium levels Disappearance of the P wave with idioventricular regular cardiac rhythm, or more often supraventricular tachycardia, occurred in the patients showing the highest potassium values

Since hypocalcaemia and hypermagnesaemia occurred in all hyperkalaemic groups, the effect of these factors on the electrocardiographic changes on the Q—T duration and the P—Q interval in particular, had to be analysed The average magnesium level rose with rising potassium values, whilst both low and almost normal calcium values were noted at all potassium levels Therefore, in fig 1 the calcium and magnesium values in the various hyperkalaemic groups are correlated with Q—T_c In the lowest hyperkalaemic group and in the group with potassium over 8 mEq/l the correlation between hypocalcaemia and prolonged Q—T_c is poor On the other hand, in the groups with potassium values between 7 1 and 8 mEq/l a prolonged Q—T_c duration seems to correlate fairly well with decreasing calcium

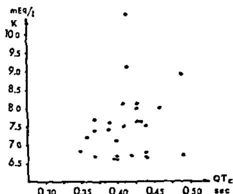


Fig 2 The relationship between serum potassium and the Q T_c interval in this series See tables I—III and text.

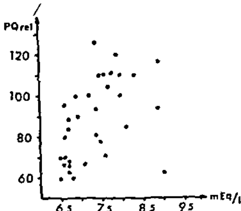


Fig 3 The influence of hyperkalaemia on the relative P Q interval (PQ_{rel} see text)

values It appears that hypermagnesaemia shows a better correlation with prolongation of Q—T_c than hypocalcaemia

In fig 2 the potassium values of all the present patients are plotted against Q—T_c There is a certain tendency towards a prolongation of Q—T_c with rising serum potassium, but dispersion is wide

In fig 3 all potassium values are shown in relation to the relative P—Q interval The latter in accordance with Ashman and Hull (2), is the ratio of the P—Q interval to the maximum normal P—Q interval (PQ_{max}) at the same frequency An almost linear increase of the relative P—Q interval with rising potassium values is observable This is in contrast with the correlation between the magnesium level and the P—Q interval, which shows a wide dispersion (fig 4)

Both metabolic acidosis and hyponatraemia are frequent in acute tubular necrosis and also occurred in the present cases but neither the blood pH nor the

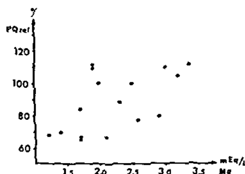


Fig 4 The influence of the serum magnesium level on the PQ_{rel} (see text) in this series

serum sodium level differed in the various hyperkalaemic groups Hence these factors showed no correlation with the electrocardiographic changes observed

Discussion

Hyperkalaemia in uraemia is to be regarded as a clinical syndrome in which not only the serum potassium level is of significance Disturbances in the calcium and magnesium balance as well as changes in the sodium metabolism also have to be taken into account Disturb-

cells into the extracellular space, with ensuing temporary hyperkalaemia. The extracellular potassium level thus seems to be decisive for the electrocardiographic changes and for the severity of the potassium intoxication, as has already been established by previous investigators (6).

We, too, have observed two cases of severe hypokalaemia in which signs of hyperkalaemia were present in the form of high T waves in the ECG. The mechanism in these cases seems to have been a rapid release of potassium from the intracellular to the extracellular space in the heart muscle, brought about by grave respiratory or metabolic acidosis.

Summary

The electrocardiographic changes associated with hyperkalaemia over 6.5 mEq/l were studied in 40 patients, 31 of whom had acute renal insufficiency whilst nine suffered from chronic renal disease. Furthermore, the effect of hypocalcaemia and hypermagnesaemia on the electrocardiographic pattern was analysed at different potassium levels. Electrocardiographic signs of hyperkalaemia were present in 27 cases (67.5 %), which is in agreement with certain other reports. At potassium levels over 8 mEq/l electrocardiographic signs of hyperkalaemia were invariable present, whilst only nine out of 15 patients with serum potassium values between 6.5 and 7 mEq/l showed such signs. Hypermagnesaemia seems to accentuate the effect of hyperkalaemia in the electrocardiogram, in particular on the lengthening of the Q—T_c duration, less on the prolongation of the P—Q interval. Hypocalcaemia occurred at all levels of hyperkalaemia, but the correlation with prolonged Q—T_c duration was poorer than the expected.

The complex character of the hyperkalaemic syndrome associated with uraemia is emphasized. Disturbances of all the various electrolytes as well as changes in the acid-base balance are relevant to evaluation of the electrocardiographic pattern. Hypotonia and cardiac hypertrophy may mask the hyperkalaemic changes of the ST segment. The relationship between the intracellular and extracellular potassium concentration in the myocardium is also important. The extracellular potassium concentration seems to be decisive for the development of the electrocardiographic changes. Rapid changes in the ratio extracellular/intracellular potassium probably also contribute to the development of temporary electrocardiographic changes.

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A Double-blind Trial of Long-term Anticoagulant Treatment after Myocardial Infarction

By

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After 1960 increasing opposition to long-term anticoagulant treatment for coronary thrombosis in our own as well as in other countries (4, 8, 14, 16, 18, 26) called for re assessment of the value of treatment, given for an unlimited period to a steadily increasing number of patients controlled at our Thrombosis Service The value of a treatment given for longer than one year had become especially doubtful Hence in 1963 we decided to undertake a double blind clinical trial including all our patients treated for longer than one year after cardiac infarction Fortunately the condition considered crucial for successful anticoagulant treatment i e effective hypocoagulability, could be achieved through the choice of a suitable drug (7 10 29) and particularly through the facilities put at our disposal by the Netherlands Thrombosis Service (20, 22 23) This paper is the final report of the trial

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Material and methods

In Jan 1964, 417 patients treated with coumarin drugs for more than one year after a clinically and electrocardiographically proven cardiac infarction were being cared for by the Thrombosis Service of Leyden Before grouping 150 of these patients were excluded from the study for the reasons indicated in table I

No males younger than 45 or older than 75 years and no females were included in the study the number of the latter being too small to satisfy requirements for separate statistical evaluation Immobilized patients were not admitted because when not treated with coumarin drugs these patients are known to run a high risk of thromboembolic complications

The remaining 267 patients all of whom had proven their willingness and suitability for treatment during the year(s) before admission to the study were divided into three age classes 45—54 55—64 and 65—74 years The patients in each class were subdivided into two chronological groups by alternating allocation according to the date of infarction, i e of admission to the Thrombosis Service The decision as to

TABLE I. Numbers of patients excluded from the trial, arranged according to reason for exclusion

Reason for exclusion	Number of patients excluded: Before grouping	After grouping: Phenprocoumon	After grouping: Placebo	After grouping: Total
Sex (women)	62			62
Age < 45 years	19			19
> 75 years	34			34
On coumarin drug other than phenprocoumon	1			1
Hypertension (> 200 > 120)		2		2
Immobilization	20	1	4	25
Atrial fibrillation		1	3	4
Malignant tumour	1			1
Other medical reasons	4			4
Adequate supervision not feasible	2			2
Psychological reasons	7			7
Inappropriate inclusion		1	3	4
Death		1	1	2
Total	150	6	11	167

TABLE II. Data concerning comparability of the two groups of patients treated with placebo and phenprocoumon, respectively

	Phenprocoumon				Placebo			
	45–54	55–64	65–75	Total	45–54	55–64	65–75	Total
Number of patients	34	57	37	128	31	60	31	122
Mean age	50.5	59.4	67.5	59.4	50.35	59.4	69.1	59.6
Mean pre-trial anticoagulant period, mos	35.4	37.0	46.8	39.4	39.2	39.3	44.7	40.6
Diabetes mellitus	2	1	1	4	0	1	0	1
Hypertension 180 > 100	3	8	4	15	1	7	2	10
Re-infarction during pre-trial anticoagulant period	1	0	0	1	1	2	0	3
Re-infarction as indication for long-term treatment	3	6	4	13	0	5	1	6

which group should be treated with placebo was made by lot for each class separately. As a result of this procedure 134 patients would have been treated with phenprocoumon and 133 with placebo. However, re-examination of the patients revealed four inappropriate inclusions (two with insufficient evidence of cardiac infarction

TABLE III Comparability of the two groups of patients according to length of the pre trial anti coagulant treatment

	Length of anticoagulant treatment before trial (mos)								
	12—	24—	36—	48—	60—	72—	84—	96—	108—120
Phenprocoumon	39	23	23	16	13	10	2	1	1
Placebo	36	24	19	18	17	5	3	—	—

and two females), two patients suffering from severe hypertension, and four cases of atrial fibrillation Two patients died and five patients became bedridden between classi fication and the start of the trial, resulting in a total of 17 drop-outs (columns 2 and 3 of table I) Hence, 250 patients finally entered the trial 122 patients in the placebo group and 128 patients in the phenpro- coumon group The period during which the patients entered the trial lasted from the end of March until the beginning of May 1964

The criteria applied for comparability of the two groups are indicated in tables II and III Table III which gives the number of patients in the two groups according to the length of the pre treatment period further illustrates the similarity of the two groups

At the start of the trial all patients were told that they would be treated with a new preparation similar in its action to phen pro oumon The tablets given us by F Hoffman—La Roche Basel were distributed by the Thrombosis Service The placebo tablet consisted of saccharum lactis The phenprocoumon and placebo tablets were identical in shape and colour To prevent mistakes the letter M was not printed on the placebo tablets The therapeutic regimen was the same in patients of both groups except for the anticoagulant drug Vitamin K1 prophylaxis for tooth extraction was given in all patients Two of us (AH and NF) were in charge of the dosage of phen procoumon and placebo The dose of the coumarin drug was prescribed on the basis of the results of the coagulation test the dose of placebo according to that of phen procoumon in the preceeding year As a security check a calendar with indication of the daily dosage was mailed to the patients after each coagulation test The patients were asked to cross out each dose of the medicament as soon as it was taken The mean daily dose of phenprocoumon was 2 8 mg

Attending physicians—general practitioners and specialists—had given their full consent to the trial They were not informed about the kind of treatment and they received instead of the result of the coagulation test a monthly reminder that the patient had been checked for the long term anticoagulant treatment In case of re infarction other kinds of important cardiovascular deterioration appearance of dysbasia intermittens cerebro vascular accident, or venous thrombosis and lastly, in case of bleeding the attending physi cian contacted one of us (EAL) The decision as to the type of treatment was made before the patient s status in the trial was known if the decision was new indication for anti coagulant treatment he was removed from the trial

The main criteria for determining the value of the therapy were death rate and rate of re infarction. The diagnosis re infarction based upon clinical electro- cardiographical and if available biochemical investigations has been made by two dif ferent cardiologists (FK and LMvD) In addition the rate of other cardiovascular complications was determined Finally 14 months after the beginning of the trial an investigation into complaints was made

At the end of Aug 1965 the trial was

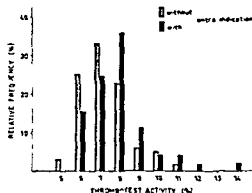


Fig 1 Diagram of intensity and stability of hypocoagulability as measured by means of thrombotest activity The values are grouped in classes of 1 % Solid columns represent values found in patients without contra indications and hatched columns those found in the patients with "relative" contra indications (see text)

TABLE IV Illustration of intensity and stability of treatment 696 thrombotest per centages (a representative sample of the phenprocoumon group) were classified as shown 94 1 % of the values lay below 15 % and 87 3 % between 5 % and 15 %

Thrombotest value-classes (%)	No of values (class %)
< 5	6 8
5—10	68 2
> 10—15	19 1
> 15—20	4 0
> 20—25	1 2
> 25—30	0 4
30	0 3

finished All patients were switched to regular phenprocoumon therapy Tablets were no longer supplied gratuitously by the Thrombosis Service the patients had to buy them on prescription.

The coagulation check consisted of thrombotest performed on venous blood In cases with no contra indication we tried to attain values of thrombotest within the range of 5 %—10 % whereas in cases with relative contra indications (almost 50 % of the patients displayed relative contraindications such as age > 65, hypertension, anamnestical cerebrovascular accident cured gastric or duodenal ulcer) the corresponding range was 7 %—13 %

Fig 1 and table IV demonstrate the intensity of the coumarin effect as assessed by thrombotest For each of the 116 patients still in the trial after one year of treatment six of the 12—18 thrombotest values obtained during the first 12 months of the trial (= a total of 696 values) were collected by taking the values of the first check of every 2 month period The logarithm of these values was used for the calculation The anti log of the mean of the six logarithmic values was converted into % TT and considered to be representative of the level achieved in the individual In fig 1 the individual average values for these 116 patients are distributed into classes of 1 %

As fig 1 shows the 59 patients without contra indications were more intensively treated than the 57 patients with relative contra indications The overall mean thrombotest activities were 7.5 % and 8 2 % respectively The standard deviations of the individual means were 1.25 % and 1 48 % respectively indicating that the stability of hypocoagulability was somewhat better in patients without contra indications

In addition, the variation of thrombotest activity in individual patients was calculated from the 116 × 6 values the standard deviation of the individual thrombotest percentages was about 2 9 %

Table IV in which the 696 thrombotest values are classified according to activity in classes of 5 % illustrates the intensity and stability of the treatment

The thrombotest values of the eight sudden deaths two re infarctions, and two important cardiovascular deteriorations during phenprocoumon treatment were analyzed separately They do not differ from those found in the other patients either as to

TABLE V Classification of bleeding complications according to treatment c q severity

	Phenpro-coumon	Placebo
No change in dosage	11	6
Temporary lowering of dosage	3	—
Administration of vitamin K_1	[1]3	[2]1
Blood transfusion	—	—

[1] Two nacrohaematurias one subarachnoidal bleeding
[2] Lethal intracerebral bleeding

intensity or as to stability of the hypocoagulability

The adequacy of the treatment was further assessed by calculating the bleeding frequency tables V and VI show these results

Results

Immediately after the beginning of the trial a transitory increase of complaints in patients of both groups was observed There was however no accumulation of cardiovascular complications either in the placebo or in the phenprocoumon group during this period The results of the trial are summarized in table VII Details of the 16 sudden deaths, 14 re infarctions and 15 other cardio vascular complications are given in tables VIII IX and X

Besides the patients referred to in tables IX and X, all of whom were switched from placebo back to phenprocoumon, three other patients had to be removed from the trial, two suffering from venous thrombosis while on placebo and one displaying a sub arachnoidal bleeding during phen procoumon therapy

TABLE VI Classification of bleeding complications according to localization

Localization	Phenpro coumon	Placebo
Cutaneous	5	0
Nasopharyngeal	7	5
Intestinal	1	1
Urogenital	2	0
Intracranial	1	1
Subconjunctival	1	0

TABLE VII Summary of the results

	Phenpro coumon	Placebo
Number of patients		
April 1964	128	122
Aug 1965	112	81
Years of exposure to risk	168	138
Sudden death	8	8
Re infarction	2	[1]12
Important cardiac deterioration	1	[1]7
Dysbasia intermittens	1	4
Cerebrovascular accident	—	[1]2
Venous thrombosis	—	2
Total cardiovascular complications	12	35
Severe bleeding complications	[2]1	[3]1
Number of drop-outs	5	7

[1] One death
[2] Subarachnoidal bleeding with complete recovery
[3] Lethal intracerebral haemorrhage already tabulated under cerebrovascular accident

TABLE VIII Data concerning patients who died suddenly The pre treatment period is the time of phenprocoumon treatment before the start of the trial

Pats	Age	Pre treatment period (mos)	Treatment period (mos)	Cardiac symptoms before death
Phenprocoumon				
HCGT	61	15	2	No
JGS	55	29	5	Yes
BG v M[1]	52	79	9	No
C v R	64	50	9	No
WJ	55	52	11	No
HW	61	21	12	No
JJT	54	45	16	No
PJ v Z	53	22	16	No
Placebo				
J d H	71	24	25/30	Yes
KR v R	51	15	2	No
A v d V	51	64	6	Yes
JK	56	22	8	Yes
CSW	72	72	9	Yes
R v D	57	65	12	No
J v K	62	18	13	No
JZ	52	77	14	No

[1] Diabetic

Two patients one treated with phenprocoumon and the other with placebo who suffered a minor cerebrovascular accident that according to the consulting neurologist was neither an indication nor a contra indication for anticoagulant treatment remained in the trial The same holds for a patient with signs of retinal ischaemia while on phenprocoumon

The distribution of all cardiovascular accidents (deaths re infarctions and other cardiovascular complications) over observation time and age groups is shown in fig 2 Obviously there is no correlation between incidence and observation time in either the phenprocoumon or the placebo patients This may be seen even more clearly from fig 3 in which the incidence/observation time correlation is depicted cumulatively On the other hand, there seems to be a correlation between incidence and age during the 16 1/2 months of observation, 18 % of the patients of the youngest group displayed a cardiovascular accident whereas 15 % of the middle aged and only 6 % of the elderly patients did so Statistiscally, this decline is significant at a 5 % level in the placebo group whereas no clear decline is to be seen in the phenprocoumon group

Of the 25 patients suffering from re infarction or some other cardiovascular deterioration who were put back to phenprocoumon, two died from cardiac

TABLE IX Data for patients suffering from myocardial infarction

Pats	Age	Pre treatment period (mos)	Treatment period (mos)	Criteria Clin	ECG	SLDH
Phenprocoumon						
P d K	55	45	4	+	+	—
N v S	61	14	12	+	+	+
Placebo						
HC	58	66	5/30	+	+	—
WV	60	70	1	+	+	—
WS[1]	59	63	1	+	+	—
J d W	64	31	3	+	+	−
ANM	49	43	7	+	+	—
GLJK	56	22	9	+	+	+
PS[1]	47	17	8	+	+	−
WMH	47	24	10	+	+	−
J v W	49	37	12	+	+	−
JZ	53	45	13	+	+	—
WH	61	52	13	+	+	−
N v R	61	28	14	+	+	—

[1] Hypertension
[2] Patient confined to home died on 7th day after infarction
[3] Diabetes
— = SLDH not investigated

insufficiency None displayed another cardiovascular accident during the further observation period until the end of the trial On conventional statistical treatment *of the results in table VII, the* difference between the 33 cardiovascular accidents occurring in the placebo group (the two venous thromboses were excluded) and the 12 accidents occurring in the phenprocoumon group is significant at a 0 1 % level for recurrences (12 2) and other cardiovascular complications (13 2) the differences are significant at a 5 % and 1 % level, respectively However, with the statistical approach applied by Bjerkelund (2) the differences both for recurrences and for other cardiovascular deteriorations are significant at a 1 % level (P recurrences < 0 005 P cardiovascular deteriorations < 0 001) Bjerkelund s approach takes into account the fact that the size *of the placebo group diminishes more* rapidly than that of the phenprocoumon group, the total time of treatment (which can be defined as time of exposure to risk) for the placebo and the phenprocoumon groups amounted to 138 and 168 patient years respectively

Finally, in order to obtain more comparable figures the rate (%) of the different types of accidents was calculated by dividing the number of accidents by the time of exposure to risk (in years), the resulting value being multiplied by 100 Table XI gives the results

TABLE X Data for patients suffering from other cardiovascular complications

Pats	Age	Pre treat ment period (mos)	Treat ment period (mos)	Cardiovascular complications	Remarks
Phenprocoumon					
CJ v d E[1]	56	24	14	Progressive dysbasia intermittens	Sympathectomy
CB	66	75	16	Progressive angina pectoris	Infarction not proven
Placebo					
WMP	65	41	2	Progressive angina pectoris	Confined to bed dubious ECG changes
RW	54	79	3	Acute cardiac insufficiency	Admitted to hospital dubious ECG changes died 3 months after attack
JR[2]	59	50	3	Prolonged attack of angina pectoris	Confined to bed infarction not proven
CG	71	16	3	Cerebrovascular accident	Lethal intracerebral haemorrhage
PJ v H	62	12	8	Cerebrovascular accident	Complete recovery
W v d K	61	40	8	Acute cardiac insufficiency	Admitted to hospital infarction not proven
CK	64	36	8	Acute stenocardia	Admitted to hospital infarction not proven
PZ	50	69	8	Progressive dysbasia intermittens	Vascular prosthesis
JAZ v d L	49	69	8	Progressive dysbasia intermittens	
WK	54	22	10	Progressive dysbasia intermittens	Sympathectomy
FHTHJ	56	22	11	Progressive angina pectoris	Confined to bed checked at home for 4 weeks
TJL	64	54	12	Progressive angina pectoris	Confined to bed dubious ECG changes
W v B	63	18	16	Progressive dysbasia intermittens	Sympathectomy

[1] Diabetic
[2] Hypertension

The death rate, undoubtedly the main criterion for the success of anticoagulant treatment, was 4 8 % (8/168 × 100) in the phenprocoumon and 7 2 % (10/138 × 100) in the placebo group If we add to the length of exposure to risk the ob

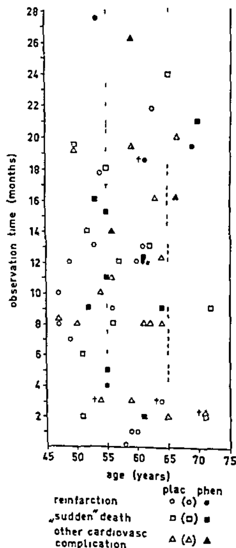


Fig 2 Synopsis of cardiovascular accidents related to time of treatment (ordinate) and age (abscissa) After the end of the trial (horizontal dotted line) complications occurring in patients belonging to the former placebo group are represented by punctuated signs (* = same patient)

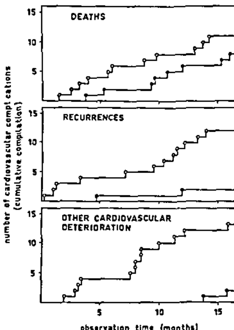


Fig 3 Occurrence of cardiovascular complications related to observation time (cumulative compilation) In the figure depicting cardiovascular death rate there are included besides the 16 sudden deaths, the two patients put back on phenprocoumon shortly before their death as well as the lethal cerebral hemorrhage in a patient belonging to the placebo group

servation time of patients returned to phenprocoumon (14 years for placebo patients and two years for phenprocoumon patients) the respective figures become 4 7 % (8/170 × 100) and 7 2 % (11/152 × 100) There appears to be no significant difference between the death rate in the two groups considered in either way The re infarction rate however is distinctly higher in the placebo group than in the phenprocoumon group (8 7 % against 1 2 %) the highest difference found in the youngest age group (15 % against 0 %) A similar conclusion holds for the difference in incidence of other cardiovascular complications

For the two younger age-groups taken together the rate of the sum of re infarctions and other cardiovascular

TABLE XI Incidence of cardiovascular accidents calculated for the total material and for the three different age groups classified according to type of accident Incidence=no of accidents/yrs of exposure to risk in %

	45—54	55—64	65—75	All patients
Placebo				
Years of exposure to risk	33 5	69	35 5	138
Mortality (%)	9	4 3	5 6	7 2
Recurrence (%)	15 0	10 1	0 0	8 7
Other cardiovascular complications (%)	12 0	10 1	5 6	9 3
Phenprocoumon				
Years of exposure to risk	46 5	73	48 5	168
Mortality (%)	6 5	6 9	0 0	4 8
Recurrence (%)	0 0	2 8	0 0	1 2
Other cardivascular complications (%)	0 0	1 4	2 1	1 2

TABLE XII Number of cardiovascular complications classified according to length of pre trial anticoagulant treatment

	Length of anticoagulant treatment before trial (mos)						
	12—23	24—35	36—47	48—59	60—71	72—83	84—120
Re infarctions	3 (1)	3	4 (1)	1	3	—	—
Deaths	7 (3)	3 (1)	1 (1)	2 (2)	2	4 (1)	—
Other cardiovascular complications	5	1 (1)	3	2	2	2 (1)	—

Numbers within brackets refer to patients belonging to the phenprocoumon group

TABLE XIII Evaluation of complaints as expressed by patients without definite cardiovascular complications The feeling of improvement occurred significantly more often in patients on phenprocoumon than in patients on placebo ($p<0\ 05$)

	Improved	Unaltered	Worse	Total no of pats
Phenprocoumon	34 2 %	54 4 %	11 4 %	114
Placebo	19 3 %	65 8 %	14 5 %	76

TABLE XIV Evaluation of complaints as expressed by patients put back on phenprocoumon

	Improved	Deteriorated	Unchanged	Total
Phenprocoumon				
Re infarctions	—	—	2	2
Other cardiovascular deterioration	—	1	1	2
Placebo				
Re infarctions	1	5	5	11
Other cardiovascular deterioration	2	2	6	10

complications was slightly more than nine times higher in patients on placebo (24 %) than in those on phenprocoumon (2 6 %)

Conspicuously enough there is no correlation between the occurrence of accidents and the length of the pre treatment period either for sudden deaths and re infarctions or for other cardiovascular deteriorations This becomes evident when the results shown in tables III and XII are tested together against trend in a k × 2 contingency table with approximation

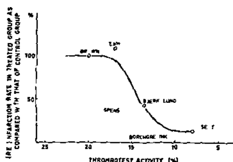


Fig 4 Tentative curve of correlation between (re)infarction rate and intensity of treatment constructed upon data published or furnished by authors of controlled clinical trials

Between July 15 and Aug 15, 1965, 190 of the 192 patients still included in the study were asked, under blind conditions about their complaints at the end of the trial as compared to those before its start, especially cardiac symptoms Table XIII summarizes the results of this interrogation. More of the patients treated with phenprocoumon than of those on placebo felt improved at the end of the trial (significant at a 5 % level test according to Yates and Cochran) Of the 29 patients who returned from the placebo group to anti coagulants because of re-infarction and other cardiovascular complications 26 were still alive in Aug 1965 Twenty five of them could be interrogated Table XIV shows the results almost one third of the patients felt definite deterioration Only three felt improved

The results of the interrogation reinforce the evidence in favour of long term anticoagulant treatment, although the patients' complaints are known to be the least objective criterion

After discontinuance of the trial although the daily number of tablets to be taken was not altered, the change in posology caused in some patients irrespective of the type of drug they had been treated with a transient increase of complaints similar to those observed immediately after the start of the trial

Patients who had been treated with placebo were checked a fortnight after the change of regimen. In most of them no important alteration in sensitivity to phenprocoumon appeared to have taken place. There were no important bleeding complications. Interestingly enough, however, as demonstrated in fig. 2, the rate of cardiovascular complications remained unaltered for about three months after discontinuance of the trial on Sept. 1: between Sept. and Dec. 1965, six accidents were observed in the former placebo group as compared with only two in the phenprocoumon group (as indicated in fig. 2, the one of these two who died from re-infarction had already suffered re-infarction during the trial). Only in the course of the following seven months of observation (until July 1966) did the rate of complications decrease: it then became similar for both groups. It should be emphasized that, during this second period of observation no patient had been lost from observation, that there had been only one drop-out (due to a gastric ulcer) and that no severe bleeding complications were noted.

Discussion

The present study was undertaken in an attempt to decide whether it is worth while to continue long-term anticoagulant treatment for more than twelve months after a myocardial infarction, a question raised by Bjerkelund at the Toronto meeting in 1961 (4). We were fully aware that only a study satisfying the criteria of both a double blind clinical trial and an adequate anticoagulant treatment could give us the answer.

From the design of our trial, described under Material and methods, it can be seen that the *conditions of a double blind trial* (13) *are fulfilled*: patients were known to be suitable for participation in the trial, allocation into phenprocoumon and placebo groups was made randomly, and the therapeutic regimen in both groups was the same except for the anticoagulant drug. However, as there was selection, i.e. exclusion of female patients, patients older than 75 and younger than 45 years, patients suffering from hypertension, patients physically unable to visit the laboratory of the Thrombosis Service, and patients with atrial fibrillation, etc., this selection could scarcely have biassed the structure of the two groups. Finally, the patients can be considered representative for Leiden and its surroundings because all the local cardiologists and internists follow the same line of treatment and refer all their patients to the Thrombosis Service for supervision of the anticoagulant treatment. With regard to the intensity and stability of the anticoagulant action (table IV, fig. 1), the conclusion seems warranted that *most of the patients had received adequate treatment*. In cases without contra-indications our aim was a stable hypocoagulability with thrombotest values between 5 % and 10 %, as can be seen from fig. 1, this was in the main achieved. In patients with relative contra-indications, coagulability was less uniformly depressed, as can be concluded from the larger standard deviation of the individual means. But in this group, too, values above 15 % were exceptional.

Under these conditions the *results*

obtained may indeed be regarded as relevant to the discussion of long term treatment after myocardial infarction

The difference in the rate of cardio vascular deaths—4 8 % in the phen procoumon group as against 7 2 % in the placebo group—is not significant There is, however, a trend in favour of anticoagulant treatment This becomes even more definite if this result is combined with the rate of cardiovascular deaths observed in a parallel trial performed under very similar conditions in 144 patients suffering from peripheral sclerosis (17) In the latter study the figures were 2 3 % (two deaths) in the phenprocoumon group against 9 5 % (eight deaths) in the placebo group Here too, no correlation could be found between occurrence of death and length of the pre treatment period This suggests that unlimited long term anticoagulant treatment in patients suffering from atherothrombosis prolongs life

More obvious and undeniable is a reduction of morbidity The difference between the two groups as to *re infarction rate and rate of other cardiovascular complications* (important cardiac deterioration dysbasia intermittens cerebrovascular accidents), if considered with the statistical approach used by Bjerkelund (2), is highly significant Phenprocoumon appears to provide a powerful protection against such events, especially in patients under 65 years of age (fig 2 table XI) Moreover the favourable effect appears to be independent of the duration of the anticoagulant treatment (table XII)

As far as we can judge from the literature such favourable results in patients treated for more than twelve months have seldom been obtained in the past This may be due to the exceptionally high intensity and good stability of hypocoagulability achieved in our patients Although a similar quality of treatment is known to be required for effective prevention of the venous thromboembolic diseases (32), its necessity is not generally accepted for the prevention of arterial thrombosis

Neglect of the specific aim of anticoagulant treatment—therapeutically effective hypocoagulability—may indeed explain the lack of agreement about whether patients with coronary heart disease benefit from long term anticoagulant treatment We therefore attempted, for the 12 controlled clinical trials thus far reported (1, 2 5, 8, 11, 12, 18 25, 27, 30, 31, 34), to correlate intensity of hypocoagulability with therapeutic success as measured by reduction of the re infarction rate observed in coumarin treated patients The results of the five studies suitable for such an evaluation (1, 2, 5 8 31) are shown in fig 4 in which the number of (re)infarctions occurring in patients while on coumarin treatment is given as percentage of the number found in the control group The intensity of treatment is expressed as grand mean of percentage values for thrombotest activity

In evaluating data concerning hypocoagulability, we took into account that activities below 50 % of normal found with the original P P method correspond to approximately 5/3 of those found with the original thrombotest (21 33) the difference being caused by preprothrombin a circulating anticoagulant appear

ing in the circulation during coumarin treatment (19)

The only investigators who seem to have reached an intensity of treatment closely resembling ours, at least as far as patients treated with phenprocoumon are concerned (15), are Clausen et al (11) These authors achieved a considerable reduction of the rate of recurrence in patients younger than 55 years limited to the first year of treatment However, the importance of this result is difficult to assess because insufficient data are given as to the comparability of the coumarin and placebo groups The results, therefore, are excluded from fig 4

The rather strong hypocoagulability instituted by Borchgrevink (5) in patients suffering from angina pectoris receiving intensive treatment with phenindione was accompanied by very favourable clinical results The mean P P activity was 19 % (= 11 4 % thrombotest activity) only about one fifth of the values exceeded 25 % P P activity (= 15 % thrombotest activity) Entirely negative results were obtained in patients treated during a supplementary period of observation when the mean thrombotest value was 19 % (6)

Authors who have achieved moderate intensity and stability of coumarin action appear to have obtained partially favourable results Bjerkelund (2 3, 4) concluded from his study that long term treatment with dicoumarol is primarily indicated in the younger age groups (< 60 years old men), and that the effect achieved during the first 12 months seems not to be lost after gradual cessation of therapy In this first report Bjerkelund gives figures from which a mean P P activity of about 23 % (= 13 8 % thrombotest activity) can be calculated (2) Detailed figures indicate reasonable stability of treatment

Similar conclusions hold for the British Medical Research Council (B M R C) trial (27, 28) in which phenindione had been the drug of choice, although it is difficult to convert prolongation of prothrombin time into percentage thrombotest, for acetone dried human brain thromboplastin, which was used by the authors of the B M R C trial, the prolongation sought, 2—2 1/2 times normal, probably corresponds to 5—12 % thrombotest percentage Unfortunately, exact figures concerning intensity of treatment are not given There is, however, the interesting finding of insufficient hypocoagulability (prothrombin time < 2 times normal) both during the trial and before death in about 50 % of the patients who died of re infarction or other cardiovascular causes, whereas in survivors this percentage was only one third The high percentage of insufficient hypocoagulability demonstrates the rather low stability of treatment achieved in the British trial

An equally positive result was obtained in the Veterans Administration (V A) study (34) treated and untreated patients differed significantly both in survival (0 05 > p > 0 01) and in re admission for cardiac infarction and congestive heart failure (p < 0 01) Prothrombin activity in treated patients seems to have been effectively reduced for with use of human brain thromboplastin over 80 % of the values were

below 20 %. This corresponds to a prolongation of the prothrombin time of more than twice the normal (for human brain thromboplastin, 20 % prothrombin activity equals a thrombotest value of about 12 %).

Aspenstrom and Korsan Bengtsen (1) conclude from their double blind study with dicoumarol and placebo that it is especially poor risk patients who benefit from long term prophylaxis. The intensity of coumarin was comparable to that of Bjerkelund, the mean P.P. value being 23 % (personal communication), which corresponds to 13.8 % thrombotest.

A less intensive anticoagulant treatment with phenindione, as applied by Seaman et al. (31), did not produce favourable results. These authors achieved a mean P.P. activity of 28 % (= 16.8 % thrombotest activity), about half of the values being higher than 25 % P.P. activity (= higher than 15 % thrombotest activity).

The completely negative result obtained with dicoumarol by Brown et al. (8, 9) accompanied a grossly insufficient anticoagulant effect, in a random sample, 67 % of the thrombotest values were found to lie between 10 %—30 % and even as much as 6 % higher than 30 %. Moreover, the high incidence of bleeding complications observed by these authors suggests a low stability of hypocoagulability. The negative reports of Lovell et al. (24, 25) and Conrad et al. (12) are irrelevant to the present discussion because the former give insufficient and the latter no information at all concerning intensity of treatment. Likewise difficult to assess are the results reported by Harvald et al. (18), because the requirement of random allocation does not seem to have been fulfilled (the placebo group is much larger than the phenprocoumon group) and no detailed figures concerning intensity or stability of treatment are given.

Convincing confirmation of the correctness of our reasoning concerning the correlation between intensity of anticoagulant treatment and the improvement (fig. 4) has been found in the report of a controlled clinical trial performed by Rozenberg et al. (30). In this trial, patients suffering from ischaemic heart disease, in whom it was attempted to maintain between 7 % and 12 % thrombotest activity, did significantly better than those within the 10 %—20 % range.

A decision on whether intensity of treatment is crucial calls for more trials performed under the conditions of our study. In our view intensity and stability of the coumarin action can be achieved easily and safely, provided that a long acting coumarin preparation is used and that the patients are carefully supervised. An institution such as the organization of the Netherlands Thrombosis Service in which the members of the staff (physicians and nurses) are fully responsible for the coagulation check and an adequate dosage of the coumarin drug (20, 22, 23) may be pre requisites.

Summary and conclusion

A double blind clinical trial of long term anticoagulant therapy after myocardial infarction was performed over 16 1/2 months at the Thrombosis Service of Leiden. Phenprocoumon and placebo were the drugs of choice. Included in

the study were 250 male out patients, aged from 45 to 75 who had been treated with phenprocoumon for at least 12 months before the start of the trial The therapeutic regimen in both groups was the same except for the anticoagulant drug

The incidence of cardiovascular deaths in the phenprocoumon group was 4 8 % as against 7 2 % in the placebo group This difference is not statistically significant The number of re infarctions and the number of other important cardiovascular complications however, were both much lower in the phenprocoumon group the difference being significant at a 1 % level The two younger age groups seem to have benefitted from phenprocoumon treatment more than the eldest group The rate of occurrence of complications was independent of the length of pre treatment with phenprocoumon

The favourable clinical results are based upon a high and stable intensity of hypocoagulability The mean thrombotest activity was 7 5 % and 8 2 % in patients without and with relative contra indications respectively the standard deviation of the individual means being 1 25 % and 1 48 % Less than 6 % of the thrombotest values were higher than 15 %

Despite such intensity of treatment the frequency and the severity of bleeding complications were low The rate of notable bleedings was roughly one per ten patient treatment years during the 168 patient years of treatment vitamin K_1 was used only three times and phenprocoumon treatment had to be discontinued only once because of a bleeding complication No blood transfusions were given and no lethal bleedings occurred (the patient who died of cerebral haemorrhage belonged to the placebo group)

A comparative study of our results and of those published earlier strongly suggests that the extent to which recurrent coronary thrombosis is prevented by anticoagulant treatment depends primarily on the intensity of hypocoagulability achieved Moderate hypocoagulability (thrombotest values 12 %—25 %) is of limited or no value whereas a sufficiently intensive treatment (thrombotest values of 5 %—12 %) appears to improve the prognosis of coronary heart disease for an unlimited period of time at least in ambulant male patients under 65 years of age The achievement of a sufficiently intensive and stable hypocoagulability may depend however on tight supervision of the patients such as is provided by the organization of the Netherlands Thrombosis Service and on the use of phenprocoumon for its conspicuously prolonged anticoagulant effect

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A Clinical Trial of Guanoxan (Envacar®) in Hypertension Resistant to Common Drugs

By

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The increase in mortality and the serious complications associated with arterial hypertension have been documented over many years (11, 25, 30)

It has been pointed out among others by Keith et al (13) and Smirk (24) that mortality rises with increased blood pressure and severity of retinopathy

Active and effective treatment of hypertension can prolong life In the last decade in particular, with the appearance of a number of potent hypotensive agents emphasis has been laid also on the early introduction of effective antihypertensive treatment in essential hypertension (3, 11, 28) Relatively advanced age and complicating diseases need not stand in the way of active therapy if due caution is exercised (10, 18)

In the milder forms of hypertension thiazide preparations have proved adequate (8, 17, 26) In more severe cases therapy with a combination of drugs is generally preferred to reduce the risk of side effects and toxic reactions (14, 23, 24) The commonest drug combinations in present antihypertensive therapy are probably thiazide and guanethidine and/or α methyldopa (29) Although there is now a wide range of combinations from which to choose, patients who have proved fairly resistant to treatment and/or have experienced troublesome side effects are still encountered These side effects may rule out a requisite increase in dosage and hence stand in the way of an effective lowering of the blood pressure

This situation prompted us to try out a new hypotensive preparation, known as guanoxan (Envacar®, Pfizer) in a series of hypertensive patients most of whom had proved resistant to therapy Guanoxan, whose chemical name is 2-guanidinomethyl 1, 4 benzodioxane sulphate, was synthesized by Augstein and Green (1) and its pharmacological properties have been investigated by Davey and Reinert (7) In laboratory animals

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guanoxan has been found to exert a peripheral adrenolytic action, it depletes the catecholamine content of the hypothalamus, adrenal medulla and peripheral tissues and blocks the α receptors in the smooth muscle of the vessel walls Thus, guanoxan has both a central and a peripheral action and thereby differs from guanethidine

Material

The series consisted of 30 patients with hypertension Twenty eight had received previous treatment with one or more hypotensive agents but with little success either because they had little or no effect or because side reactions were too severe In two cases the hypertension had come to light only recently

The previous treatment was with different agents such as thiazid preparations guanethidine hydralazine and α methyldopa Most cases had been treated with various combinations of two or several of these drugs

Twenty of the patients were women and ten men Eighteen were in the 50—59 age group the youngest was 40 years and the oldest 67 Twenty three had essential and seven renal hypertension In seven cases the condition had been present for 6—10 years and in 12 more than 11 years The hypertension was graded according to the diastolic pressure in mm Hg as follows mild 95—110 moderate 111—125 severe 126—140 gross more than 140 Ten patients were assigned to the moderate group 19 to the severe and gross groups

Changes in the eye fundus were graded according to the system of Keith et al (13) seven patients had grade III—IV 16 grade II, and seven grade I changes Twenty four patients had ECG features suggestive of left ventricular strain in the others the pattern was normal Four patients had radiological cardiac enlargement Five patients had impaired renal function with a plasma creatinine level of 1 3 mg—2 6 mg/100 ml in the remaining 25 levels no higher than 1 2 mg/100 ml were recorded Other conditions related to the hypertension were found in 18 patients, two of them showed symptoms of decompensation and three had angina pectoris

Selection of cases

Patients were selected on the basis of their hypertension, without regard to other complicating diseases The first 28 of the 30 patients were chosen since they were found to be resistant to conventional antihypertensive therapy With four exceptions only those with a diastolic pressure of more than 110 mm Hg after a week in the hospital were included Two of the exceptions had a strong hereditary trait for hypertension all four had eye fundus alterations and pathological ECG features and, at previous out patient checks a high diastolic pressure

Methods

All the patients in the series were admitted to, and examined at, the Department of Internal Medicine Vastervik The average stay in hospital was 25 days On admission all hypotensive agents and sedatives were withdrawn Five patients who were found to be agitated and nervous were placed or left on a light sedative chlordiazepoxide (Librium® Hoffman La Roche) Adipose patients were given a low calorie diet (800—1 000 cal /day) All patients were ambulatory throughout their hospital stay

The following tests were performed complete clinical examination renal function tests (plasma creatinine creatinine clearance repeated inspection for sediment and measurement of specific gravity and urine cultures), examination of plasma electrolytes catecholamines in the urine liver function tests, hematological examination eye fundus and ECG X ray examination of the heart and lungs and urography

B P and pulse rate were recorded every four hours for 24 hours on the second and eighth days after admission Each recording was made after about 30 min of rest in

the recumbent position, after rising and after standing for a few minutes Thereafter checks were made every morning in the recumbent and erect postures

All patients were given guanoxan orally Usually it was given in the second week after admission with an initial dose of 10 mg b d increased by 10 mg every three days until the diastolic pressure in both recumbent and erect postures was reduced to $\leq$ 100 mm Hg Throughout the period of treatment in the hospital B P and pulse were checked three times daily, at 8 00 15 00 and 22 00 hours

In some cases it was necessary to combine guanoxan with polythiazide (Renese® Pfizer) 1—2 mg/day because side effects precluded any further increase of the dose or because there was a tendency to fluid retention and increase in body weight On introducing combined therapy the dose of guanoxan was halved as soon as the polythiazide was started After adequate reduction in B P had been obtained the patients were observed for a few days at the hospital before discharge

Blood picture, liver and renal function plasma electrolytes body weight and ECG, were examined while at the hospital once a week and then at each out patient visit After discharge the patients were checked at first every fortnight and then monthly or bimonthly Examination of the patients both in the hospital and afterwards was performed by the same physician At out patient examinations B P and pulse were recorded as described above

The duration of treatment with guanoxan varied between 5 and 16 months with a mean of 13 months

Results

The hypotensive effect was assessed in accordance with the diastolic pressure in mm Hg as follows, good control recumbent $<$ 111 and erect $<$ 101 poor control erect $>$ 120 moderate control (between good and poor) recumbent and erect but at least 20 mm Hg lower than before giving guanoxan

TABLE I Indication for combination therapy with polythiazide in 15 patients

	No of pats
Resistance to guanoxan	0
Reduction of side effects	6
Enhanced antihypertensive effect and reduction of side effects	7
Weight gain and/or cardiac failure	2

TABLE II Statistical comparison of diastolic B P during previous treatment (1) after one week in the ward (2) and at the end of observation (3)

	Comparison 1 against 3		Comparison 2 against 3	
	Lying	Standing	Lying	Standing
No of patients	[1]27	[1]25	26	26
Mean difference (mm Hg)	34 8	43 6	20 0	28 5
Standard deviation	3 7	3 9	3 3	3 5
Significance P $<$ 0 1%	*	*	*	*

[1] The material lacks 3 5 and 4 values respectively

TABLE III Maintenance dose of guanoxan in 27 patients

	Guanoxan alone (mg)	Guanoxan and polythiazide (mg)
Mean dose/day	65	58
Range/day	20—160	10—160

TABLE IV Side effects

	Transitory[1]	Persistent[1]
Drowsiness weakness	6	6
Postural hypotension	8	5
Diarrhoea a/o bowel urgency	11	1
Exercise hypotension	3	4
Headache	6	1
Nausea	7	2
Nasal stuffiness	3	1
Vomiting	3	0
Drug fever	0	1
Angina pectoris	6	1
Sexual disturbance	0	1
Itching	1	0

[1] No of patients with side effects total 28 transitory 19 persistent 9

In all but one of the 23 patients who *started treatment with guanoxan alone*, the results were classed as good or moderate In 15 of them it was necessary, for various reasons, to incorporate polythiazide after 1—10 months of therapy In 13 cases, combined therapy was introduced to decrease the side effects and in seven of these it was needed also to increase the hypotensive effect In two cases polythiazide was also given because of an increase in body weight (table I) Four patients were already taking this drug when guanoxan treatment was begun Two of them were receiving it for cardiac decompensation and in the other two guanoxan was also given because the B P could not be controlled with polythiazide alone Guanoxan was withdrawn in three cases because of drug fever, cerebral thrombosis and cancer, respectively The eight patients who, at the end of the observation period, were still receiving guanoxan alone, were assigned to the 'good control' group Of the 19 patients receiving combined therapy, ten showed moderate results and nine good None of the patients proved resistant to treatment

In respect of diastolic pressure, statistical comparison between the values in previous and present treatments, and between the value after one week at the hospital without therapy and the present value, disclosed highly significant differences (table II)

The mean doses of guanoxan alone and when combined with polythiazide were 65 mg and 58 mg, respectively (table III) In some cases an increase in the dose of guanoxan was necessary at the first ambulatory follow up There after the maintenance dose could be kept constant and in some cases there was even a reduction No tendency to increased tolerance was observed

The changes in the eye fundus were checked at the end of the observation period in 13 patients with more advanced retinopathy In ten patients the changes showed a regression of 1 or 2 grades, and in three cases they were unchanged

In 18 of the 24 patients there was a

regression of ECG changes over the period of observation, suggesting effective control of B P In five cases there was reduced renal function with elevated plasma creatinine before treatment was begun, one case of cardiosclerosis, with a pre treatment level of 1 3 mg/100 ml, had a level of 1 0 mg/100 ml at the end of the observation period In a man of 60 with severe diabetes mellitus the original value of 1 2 mg/100 ml has since increased to 1 7 mg/100 ml This patient was given combined therapy with guanoxan and polythiazide None of the other patients showed appreciable changes in renal function

Five patients had retired from their employment on grounds of ill health before guanoxan was introduced, the rest have been working normally since discharge from the hospital

Side effects

As with other potent hypotensive agents the frequency of side effects was high Only two of the 30 patients were without side effects during the whole treatment Side reactions were usually mild and transient and appeared during the first month of treatment (table IV) Most patients had two or more symptoms simultaneously Side effects usually appeared 30—60 min after taking the morning dose The most common although not very troublesome, was a general feeling of fatigue and weakness, often accompanied by headache or mild malaise The most troublesome were periodic diarrhoea and/or urgency of defaecation occasionally combined with severe malaise and vomiting These disorders were usually of short duration and could sometimes be controlled with antidiarrhoea agents and dieting although in some cases the dose had to be temporarily or permanently reduced Orthostatic dizziness was fairly common but dizziness during effort was rare In no cases were there syncopal episodes One patient spoke of sexual disturbance, but no consistent enquiry was made in this respect

There were residual disorders in nine out of 27 patients Six have complained of mild general tiredness, three of mild orthostatic symptoms which do not interfere with their work, one of nasal stuffiness and one of difficulty in ejaculation

Toxicity

Guanoxan was given alone, or with polythiazide in doses of up to 160—240 mg daily for 5—16 months without any evidence of haematological or biochemical toxic effects In three cases occasional elevations in SGOT and SGPT levels were noted but these had returned to normal by the time of the next check

Complications

Guanoxan was withdrawn in three cases One of them a 50 year old woman, reacted with drug fever to a dose of 20 mg after five days of treatment Renewed attempts with guanoxan therapy after one week's withdrawal again produced the febrile reaction The second case was a woman of 65 with hypertension of 12 year's standing severe ECG and eye fundus changes and signs of cerebral arteriosclerosis A reduction in the B P obtained with a dose of 10—20 mg was

accompanied by a mild cerebral vascular accident, with temporary expressive aphasia, facial paresis and slight hemiplegia A third case was withdrawn from treatment after one month for reasons unconnected with guanoxan, this was a 59 year old man who died of primary carcinoma of the liver with generalized metastases

Discussion

It is evident from animal experiments (7) that the pharmacological mechanism of action of guanoxan is many sided, but which of the various modes is responsible for the reduction in blood pressure in man is still not entirely clear (14, 15, 16 18, 19, 20)

When first given in small doses there is often an initial rise in B P (10—20 mm Hg), possibly due to the release of catecholamines but continuation of therapy produces a reduction, initially of the diastolic pressure, in both recumbent and erect postures The greatest drop occurs standing and is usually accompanied by an increase in the pulse

On administration of guanoxan the B P falls after 24 hours, although the maximum effect is not attained until after 48 hours When guanoxan is withdrawn its effect persists for up to 48 hours, after which the pressure begins to rise again

In spite of failure to therapy reported earlier in all but two of the 30 patients of the present series there was a reduction in B P on introducing guanoxan, either alone or with polythiazide in all cases This reduction was classed as good in 17 cases (57 %) and moderate in ten (33 %) In two cases the agent was withdrawn because of side effects

The hypotensive effect of polythiazide, like that of other thiazide preparations, is due not only to the reduction in plasma volume, but chiefly to a reduction of the peripheral resistance resulting possibly from an effect on the vessel wall (8, 17, 26, 29) From the present study, as well as from several others, it is evident that polythiazide potentiates the hypotensive effect of guanoxan (6, 15, 20 22, 27) The addition of polythiazide gives a further decrease within 24 hours The initial response can then give rise to a marked orthostatic reaction

In its clinical and pharmacological properties including side effects, guanoxan seems to closely resemble guanethidine, and the pattern is also found with the combined treatment According to Veldeman s (27) comparative investigation on out patients it would seem however, that guanoxan is usually more potent than guanethidine and elicits troublesome side effects less frequently This is consistent with the results of the present study and that of Peart and Mac Mahon (20)

The frequency of side effects might be considered on the high side but it should be borne in mind that it was the mild, transient disorders that predominated The untoward reactions usually appeared during the first month of treatment and then usually disappeared spontaneously, even though the dose was maintained As a rule side effects seem to occur irrespective of the dose in the individual case Postural hypotension,

malaise diarrhoea and urgency of defaecation can be particularly troublesome Since these reactions always occur in the morning usually 30—60 min after the morning dose, they can be predicted and hence controlled by the patient In the case of fatigue during the morning the symptoms can be avoided by postponing the main dose until the evening The more severe side effects would seem to be avoidable by careful choice of the dose of guanoxan and/or by combined therapy with polythiazide In the case where drug fever had occurred it was necessary to discontinue guanoxan administration In fact the majority of the patients declared spontaneously that they felt much better than before guanoxan, many felt clearer in the head, lighter and less anxious than for years Such subjective evaluations should be treated with reserve, their favourable nature perhaps being in part due to the slightly sedative effect of guanoxan (7)

It is impossible to evaluate whether the cerebral accident in a 70 year old patient was caused by the therapy or came spontaneously

Apart from orthostatic dizziness none of the side effects could be ascribed to the incorporation of polythiazide In fact it seems that side reactions tended to appear when guanoxan was given alone and diminished, or even disappeared completely when combined therapy was introduced and the dose of guanoxan decreased For 15 of the 23 patients who were first put on guanoxan alone, combined therapy with polythiazide was subsequently adopted This should be regarded as an attempt to avoid side effects during the period of treatment, rather than indicating a poor response to guanoxan alone

The toxicity of guanoxan would seem to be extremely low The occasional, transient increase in SGOT and SGPT levels might be ascribed to an impairment of liver circulation due to a drop in B P but there were no simultaneous changes in the serum bilirubin thymol turbidity or alkaline phosphatases

In a few cases there was a temporary elevation of the level of creatinine in the plasma One patient with severe diabetes showed a permanent elevation on combined therapy but this may have been associated with the underlying disease In another case of combined therapy there was temporary glycosuria but the glucose load was normal

Summary

Guanoxan was tested alone, or in combination with polythiazide over an average period of 13 months in a series of 30 patients with hypertension, 28 of which have been resistant to other forms of antihypertensive therapy The diastolic blood pressure before treatment was 115—165 mm Hg The average age was 55 years

In 17 cases (57 %) control of B P with the treatment described was graded as good, i e diastolic pressure in mm Hg in recumbent position < 111 and erect < 101 In ten cases (33 %) control of B P was moderate i e diastolic pressure < 120 mm Hg and at least 20 mm Hg lower than before giving guanoxan In three patients the preparation had to be withdrawn Control of the changes in

the eye fundus in 13 patients with more advanced retinopathy showed a regression of 1 or 2 grades in ten patients, and in three status quo

The effect of the treatment was much the same in essential and in renal hypertension, the latter, however, was present in only seven patients

The frequency of transient side effects was high, but persistent ones were found in only nine out of 27 patients In one case the preparation had to be discontinued because of untoward reactions (drug fever) Because of side effects the drug should be recommended primarily for the treatment of moderately severe and severe cases of hypertension, for the same reason combined therapy is to be preferred

A suitable approach to the treatment of such cases is a trial with polythiazide or a similar agent and if a satisfactory reduction in B P is not obtained introduction of guanoxan and cautious increase of the dose

Guanoxan may be considered a suitable preparation for reducing the B P it is effective in cases that have proved resistant to other forms of antihypertensive treatment No increase in tolerance was observed

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The Valsalva Maneuver after Operation for Atrial Septal Defect

A phonocardiographic assessment of the operative result

By

ALF WENNEVOLD

The second heart sound after the Valsalva maneuver behaves differently in normal subjects and in patients with atrial septal defect (3, 5 6)

The value of the Valsalva maneuver in the diagnosis of atrial septal defect has been investigated especially by Van der Hauwaert (5 6) He also evaluated the response of the second heart sound to the Valsalva maneuver in ten patients who had been operated for atrial septal defect (6)

It is the purpose of this study to evaluate the use of the second heart sound—including the effect of the Valsalva maneuver on the second heart sound—as a simple phonocardiographic test in the clinical assessment of the operative result in patients with atrial septal defect

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Material

Thirteen patients who were operated for atrial septal defect from May 1964 to July 1965 are included in this study The age at the time of operation ranged from 7—43 years (table I)

The diagnosis was established at right heart catheterization and confirmed at operation The defect was of the secundum type in ten patients and of the primum type in three in the latter group there was a cleft mitral valve in one patient (case 11) and the defect was uncomplicated in the other two

About 1—1 1/2 years after the operation all patients underwent a new right heart catheterization the defect was found to be closed with no left to-right shunt in nine patients as determined by oxygen saturation and/or the hydrogen test (table I) In one patient (case 13) the defect was still open with a high flow rate of 2 3 in another patient (case 9) the defect was closed but a small left to-right shunt with a flow rate

TABLE I Survey of 13 patients operated for atrial septum defect

Case no	Age (yrs)	Type of defect	Left to-right shunt (flow rate)	Result of post operative catheterization	H+ used
1	19	Secundum	> 4	Closed	
2	26	Secundum	> 4	Closed	+
3	10	Secundum	4 0	Closed	
4	10	Secundum	3 4	Closed	+
5	10	Secundum	3 1	Closed	+
6	11	Secundum	2 9	Closed	+
7	43	Secundum	2 2	Closed	+
8	25	Secundum	2 1	Closed VSD present	+
9	12	Secundum	2 1	Shunt to s c v	
10	14	Secundum	2 9	Open (FR<30 %)	+
11	31	Primum[1]	3 0	Closed	+
12	7	Primum	3 9	Open (FR<30 %)	+
13	12	Primum	4 4	Open (FR=2 3)	

[1] With cleft mitral valve

H+ = hydrogen electrode VSD = ventricular septal defect
s c v = superior caval vein FR = flow rate

of 1 5 was present through an aberrant pulmonary vein to the superior caval vein In two patients (cases 10 and 12) a very small residual shunt to the right atrium was detected only by the hydrogen test (table I)

In one patient (case 8) the post operative right heart catheterization with intracardiac phonocardiography and use of the hydrogen electrode disclosed a small hitherto undetected ventricular septal defect with a left to-right shunt of less than 30 % the atrial septal defect had been completely closed

Methods

In all patients a satisfactory phonocardiogram was obtained just prior to the operation and 1—1 1/2 years after it.

A *three-channel ink jet recorder* (*Mingograf 31 B Elema Schonander*) was used at a paper speed of 50 mm/sec The second heart sound in the pulmonary area was recorded during continuous respiration and following the Valsalva maneuver (fig 1)

The Valsalva maneuver was performed after careful instruction, the patients straining maximally for 10 sec, releasing the strain rather fast and keeping quiet in apnoea for another 5—8 sec A satisfactory straining was obtained in all patients judged by the engorgement of the neck veins by the flushing of the face by the tension of the abdominal musculature, and by the change in the heart rate following the release of the strain

The intervals between the aortic component (II A) and the pulmonic component (II P) of the second heart sound were measured and analysed

Results

Pre operatively

The interval between II A and II P varied less than 20 msec in 11 patients

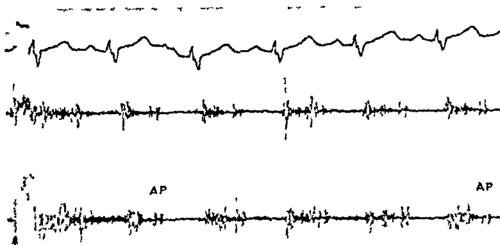


Fig 1 Pre-operative phonocardiogram recorded in the immediate post Valsalva phase from the second left intercostal space of a patient with atrial septal defect (case 6) The arrow indicates the release of the strain The split in the second heart sound is unaltered (A = aortic component P = pulmonic component Mingograf 31 B Elema Schonander recording in the 100 hz and 400 hz range Paper speed 50 mm/sec)

during continuous respiration (table II), in the two patients (cases 5 and 7) in whom the interval varied 20 msec or more it was never less than 40 msec in the expiratory phase

Immediately after release of the Valsalva strain the interval between II A and II P ranged from 40 to 80 msec (table III), and within the next few heart beats it did not decrease or it decreased only by 10 msec in nine of the patients in two (cases 1 and 4) the decrease was 20 msec and in the remaining two (cases 8 and 9) the decrease was 30 msec

Only in three patients (cases 3 8 and 13) did the interval shrink down to 30 msec

TABLE II Variation of the interval between II A and II P during continuous respiration

Case no	Pre operatively (msec)	Post operatively (msec)
1	80—70	70—40
2	50—50	70—50
3	40—30	50—20
4	50—40	60—20
5	60—40	60—40
6	50—40	60—30
7	70—40	70—20
8	40—30	60—10
9	60—50	60—30
10	50—50	50—40
11	60—60	60—40
12	50—40	60—20
13	40—30	50—30

Post operatively

In the two patients with primum defect—in whom the defect was either completely closed or who had only a minimal residual shunt (cases 11 and 12)—the splitting of the second heart sound during respiration became more variable (table II) after the Valsalva strain the

TABLE III The interval between II A and II P following the release of the strain during the Valsalva maneuver

Case no	Pre operatively (msec)	Post operatively (msec)
1	80—60	60—40
2	60—50	70—40
3	40—30	60—20
4	60—40	80—30
5	40—40	60—0
6	50—40	100—30
7	30—40	80—10
8	60—30	60—20
9	70—40	50—30
10	60—50	60—40
11	60—50	60—50
12	50—40	60—30
13	40—30	50—50

splitting in one of these patients showed a decrease of 30 msec as compared to 10 msec before the operation while the decrease was unchanged (10 msec) in the other patient (case 11) who had a cleft mitral valve not touched at the operation (table III)

In the patient with a large residual shunt (case 13) no changes occurred in the variability of the interval between II A and II P during respiration or after the Valsalva strain

In eight of the ten patients with secundum defect there was an increase in the variability of the II A—II P interval during continuous respiration (table II), in one patient (case 5) no change was noted In another patient (case 10) —the only one with a residual shunt through the atrial septal defect though very small—there was a variation of only 10 msec post operatively

After release of the Valsalva strain the II A—II P interval ranged from 50 to 100 msec in the ten patients with secundum defect (table III) With the ensuing heart beats the interval de creased by at least 20 msec in all

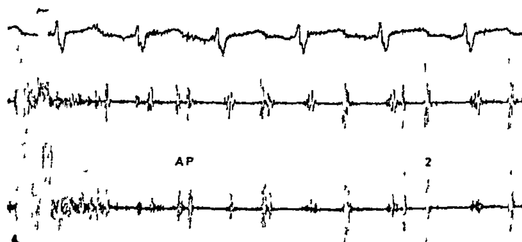


Fig 2 Phonocardiogram from the same patient as in fig 1 two weeks after successful closure of the defect The splitting of the second heart sound shortens until only a single sound is seen One year after operation the shortening of the splitting in this patient was slightly less pronounced (table III)

patients reaching 40 msec in three patients and 30 msec or less in the remaining seven

A greater decrease in the interval as compared with the pre operative findings was seen in eight patients (fig 2) while the decrease was unchanged in one patient (case 1) and even smaller in the patient with a residual shunt to the superior caval vein (case 9)

Discussion

After closure of an atrial septal defect the murmur usually diminishes and there are radiological and electrocardiographic changes towards normalization (these changes include decreases in the heart size the prominence of the pulmonary artery the vascularity of the lungs the size of the R wave in V_1 and the duration of the QRS complexes and normalization of the QRS axes) (1 2)

Such changes indicate a good operative result but they are not considered reliable enough and for a final evaluation a post operative heart catheterization is usually needed

The present study of the post-operative changes in the second heart sound should be of value in determining whether phonocardiography could add sufficiently to the clinical post-operative evaluation to enable heart catheterization to be avoided

The second heart sound is considered normal when the respiratory variation in the splitting is at least 20 msec (4) In patients with atrial septal defect there is usually a fixed splitting with a respiratory variation of no more than 10 msec (4)

The II A—II P interval is rather wide in normal subjects just after the Valsalva maneuver (3, 6) and during the next few heart beats the splitting diminishes—according to Van der Hauwaert (6) the second heart sound becoming almost invariably single or narrowly split

In patients with atrial septal defect the splitting of the second heart sound remains fixed with no or only minimal changes after the Valsalva maneuver

In eight patients in whom surgical closure of an atrial septal defect was achieved Van der Hauwaert found the normal response restored (6) Immediately after the Valsalva maneuver the splitting of the second heart sound ranged from 60 to 120 msec, averaging 88 msec, after a few heart beats the second sound became single or narrowly split—with an average II A—II P distance of 25 msec In two operated patients with residual left to-right shunts with flow rates of 1 8 and 1 3 respectively the II A—II P interval shortened after the Valsalva maneuver but never became less than 40 msec It was concluded that the Valsalva maneuver was particularly valuable in predicting the operative result in patients in whom some electrocardiographic conduction delay persisted after operation and in whom wide splitting of the second heart sound was recorded with only borderline inspiratory widening

In the present study the post operative increase in the respiratory variation of the splitting of the second heart sound compared to the pre operative value correctly indicated closure of the defect in nine patients while the fixed or nearly

fixed splitting correctly indicated a residual shunt in two patients (cases 10 and 13). Only in two patients (cases 5 and 12) was there a discrepancy between respiratory variation of the splitting and findings at heart catheterization.

The post-operative decrease in the II A—II P interval after the Valsalva maneuver correctly indicated closure of the defect in six patients. In two patients (cases 10 and 13) with a residual shunt the splitting remained fixed or nearly fixed.

But in four patients with a closed defect (cases 1, 8, 9 and 11) the II A—II P interval was unchanged or nearly unchanged, and in one patient with a residual shunt (case 12) there was a post-operative decrease in the splitting.

When both the respiratory variation of the splitting and the effect of the Valsalva maneuver on the splitting were considered, either or both values accorded with the findings at catheterization in the ten patients in whom the defect was found to be closed. Both values correctly indicated a residual defect in two patients (cases 10 and 13). In a single patient (case 12) both the Valsalva maneuver and the respiratory variation of the splitting wrongly indicated that the defect was closed, while a residual shunt—though a small one—was found at catheterization.

In conclusion, it may be said that in this study of a limited material the behaviour of the second heart sound after the Valsalva maneuver alone was not reliable enough to allow prediction of the operative result, but when also the post-operative change in the respiratory variation of the splitting of the second heart sound was evaluated, a valid assessment was achieved.

Summary

In 13 patients with atrial septal defect a phonocardiographic study of the second heart sound was performed just prior to and 1—1 1/2 years after operation.

In all patients the result of the operation was checked by heart catheterization.

The behaviour of the second heart sound after the Valsalva maneuver alone was not reliable enough to allow prediction of the operative result, but when account was also taken of the change in the respiratory variation of the splitting of the second heart sound, a valid assessment was achieved in this small material.

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Thymoma and Acute Leukaemia

By

Vagn Andersen and Henning Pedersen

In most cases of human acute leukaemia, the thymus is either normal or atrophic But it has been known for more than a century that a thymic tumour and leukaemia may occur together (6) so frequently in subsequent reports that a fortuitous coincidence is unlikely

Margolis (19) examined 20 consecutive autopsies of patients with acute lymphatic leukaemia and found four with enlargement of the thymus In 1932 Cooke (5), in an excellent review, collected 74 such cases from the literature and added nine of her own Unfortunately the illustrations and descriptions in these early publications tell little about the histological type of the thymic tumours

In the more recent literature there are scattered reports of association between acute leukaemia nearly always lymphoblastic and thymic tumour, usually of lymphoid type The cases of special interest are of course those in which there is found a thymic growth out of all proportion to the hyperplasia of other lymphatic structures But in many publications on thymomas cases with leukaemia are excluded, on the other hand the reports dealing with human leukaemia and lymphosarcoma often pay little or no attention to the thymus

The purpose of this article is to present three cases of concurrent thymoma and acute leukaemia and to emphasize that such cases are not so rare as commonly supposed The role of the thymus in the development of leukaemia in humans is discussed in the light of experimental findings in mice

Case reports

Case 1

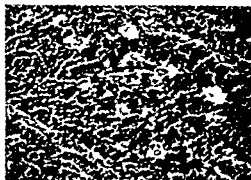
Boy born in 1956 Family and past history unremarkable

In July 1966 distended neck veins and oedema of the face were observed A few days later the boy became tired subfebrile

Submitted for publication April 11 1967



F g Ca e 1 Th moma a opera on Per a d um below



F g Case 1 P edom nantly lympho d area of thymoma H E × 180



F g 3 Case 1 Thymoma area w h sp ndle cells and rosette format on H E × 73

and d pnoe He as hosp al zed h ell d elop d sign of ompres on of the up o ena ca a There was modera e hepa omegaly and plenomegal hereas he lyn ph nod re no enlarged X ray xan na on of he he ho ed a lar rregular umour n h super or med as num compres n he ra hea h h s d lo cated ba k ard he h lar glands e e o enl rg d The Hb on en ra on as 10 2 g 100 ml he leukoc e oun 13 600/ l h 89 % cells of the l mpho ser e and the l rombo yte oun 52 000/ l

The boy as ransf rred o Surg al De partment R R gsho p talet The blood smear as dom nated by lymphoplas s A bon marro a p rate onta n d 94 % lympho cyt c cells mostly mmature Cytog ne c anal s s (chromo ome s ud s by D M Krogh Jensen) of cells from the bone marro re e led a normal chromo ome com plement Cer cal lymph node b opsy sho ed normal s ructure thout leukaem c nf l ra t on un pec f c hyperplas a of the nus re ulum cells as demons rated

A horacotom as p rformed and he h mus as found to be enlarg d lobula ed nd hard f g 1) t nf ltra d around he large e els so that only a part al remo al as poss ble

M o op c exam at on of the m ed ast nal u mour

S eral t ue blocks ere exam ned The umo r as lobulated The s ruct e a ed n d fferen t ons parts of the tumou er con posed of lympho d cells amon wh h could be seen ep hel al cells h eakly coloured nucle and ery l h PAS pos e c oplasm (f g 2) El e here h e

were seen cells somewhat bigger than lymphocytes here and there arranged in rosettes and divided by connective tissue septa Some of the cells were of spindle cell type (fig 3) The cells were polymorphous and many mitoses were observed The tumour invaded the surrounding fatty tissue

Histological diagnosis Malignant thymoma of predominantly lymphoid type

The venous stasis of the head neck and arms disappeared immediately after the operation On the fourth post operative day the blood leukocyte count had fallen to 700/μl with no immature cells The patient was transferred to Medical Department A Ten days after the operation the leukocyte count was 2 700/μl with 10 % lymphoblasts Prednisone treatment 30 mg daily and high voltage X irradiation of the mediastinum were instituted The general condition of the patient rapidly improved and the blasts disappeared from the blood Prednisone was discontinued and treatment with 6-mercaptopurine begun At discharge in Sept the physical examination was within normal limits The Hb concentration was 12 1 g/100 ml with 2 % reticulocytes leukocyte, differential and thrombocyte counts were normal In the bone marrow a few lymphoblasts could be found

The boy is now being followed as an out patient Five months after discharge he is still in remission and the superior mediastinum is not enlarged

Comment

This boy had a thymoma with thoracic inlet obstruction and a concurrent lymphoblastic leukaemia At operation the thymoma was found to be locally invasive Histologically it was of predominantly lymphoid type After subtotal thymectomy, the lymphoblasts disappeared from the blood but soon reappeared Treatment with prednisone and irradiation of the superior mediastinum induced a full clinical remission

Case 2

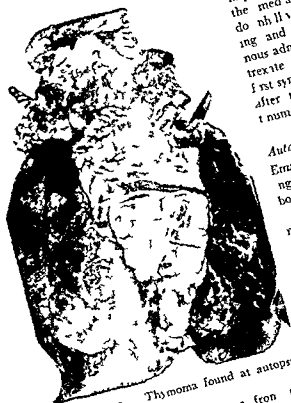
Man born in 1938 Family and past history unremarkable

In Nov 1964 the patient volunteered as a blood donor He was found to be anaemic with a Hb concentration of 11 g/100 ml X ray of the chest showed bilateral hilar enlargement whereas the superior mediastinum was normal Examination of the blood and bone marrow showed a predominance of blast cells and he was transferred to Medical Department A Rigshospitalet for treatment

Physical examination revealed an anaemic man in no acute distress without signs of bleeding His cervical lymph nodes were slightly enlarged but otherwise there was no detectable enlargement of lymph nodes or tonsils and neither the liver nor the spleen could be palpated He had a low grade fever (38—38 5 C) and the ESR was 72 mm/hr (Westergren) The leukocyte count was 30 000/μl with 51 % lymphoblasts the thrombocyte count 100 000/μl The bone marrow contained 86 % lymphoblasts establishing the diagnosis of lymphoblastic leukaemia

Prednisone treatment 40 mg daily was initiated The patient immediately became afebrile in four weeks all haematological parameters were normalized except the bone marrow smear which still contained a few malignant cells X ray of the chest was within normal limits The patient resumed his work prednisone was tapered off and treatment with 6 mercaptopurine (6 MP) 75 mg daily begun

The patient was followed in the out patient clinic He continued to feel well but in Aug 1965 the ESR began to rise again In Oct the Hb concentration decreased to 13 g/100 ml and lymphoblasts reappeared in the blood The patient complained of fever and dyspnoea Prednisone was started and the dose of 6-MP increased This relieved the symptoms but the lympho



F g 4 C a e Thymoma found at autopsy

bla ts d d no qu e d sappear fron th blood

In D he pat en began to exh b t d s ended ne k e ns h sl ght cyanos s and edem a of he fa e and ne k He had moder a el enla ged cerv cal and ax llary l mph node bu l er and spleen e e not palpable X ray of he chest sho ed a tumour in the super or med as inum h h cau ed a sl ght d loca on of the aorta to ard the left and a small tra heal mpress on be des there as h lar enlargement The bone marrow as f lled h bla t cells and here as a s eep r e in the blast cell count n the blood

Cytogenet c analys s revealed 47 chromosomes in the leukaem cells of the blood and in the bone marrow ell n 100 and 62 % of the m oses anal zed respect ely an extra chromosome as present n the group 13—15 Th s form of aneuplo dy ha earl er been des nbed n acute leukaem a (12)

6-MP as d scont nued and treatment th methotrexate begun After an in al improvement includ ng some regres on of the med ast nal tumours the cour e as do nh ll th fe er thrombocytopen c bleed ing and nfect ons in sp te of the tra e nous adm n strat on of large doses of me ho trexate In Feb 1966 15 months af er the f rst symptoms of leukaem a and t o mon hs after the tumour in the super or med as t num vas f rst noted the pat ent d ed

Autopsy

Emaciated young man Mult ple sk n bleed ngs of d fferent s z were found all o er the body

Cerv cal med ast nal and h lar lymph nodes were tremendously enlarged measur ng up to 10 cm n d ameter They ere ell demarcated of ra her soft un form con stency and the cut surface as gre h h te hout necroses

In the thym c reg on inf ltrat ng most of the anter or wall of the pericard um and aro nd the great med as nal ve el a yello h te rather f rm poorly demarca ed tumour was found On the cut surface yellow necroses up to 3 cm in diameter were found and haemorrhagic areas were seen n the ne ghbourhood of the necroses (f g 4)

The spleen as enlarged w th d sappear ance of the normal structure The k dneys h c conta ned mult ple nodules of grey sh h c colour ith a great number of haemorrha c areas The l ver was macroscop cally normal

M cro copy

The med ast nal tumour was composed of lympho d cells separated by abundant con nect e t ssue arranged in sheets of vary ng th ckness Scattered ep thel al cells er found in small clu ters or sol tary f g 5 Throughout the tumour b g n croses w h ere found l t le or no cellular react on The tumour was moderately a cular zed (f g 6) No capsule could be found and here as nva on n o the surround ng fa y s ue No normal thymic t sue as found

Bone marro lymph nodes and spleen con a ned only closely packed lympho d cell



F g 5 Case ? Thymoma of predominantly lympho d type No e scattered ep thel al cells H E × 180



F g 6 Case 2 Thymoma necrotic area H E × 18

there were no necroses Li er k dneys s prarenal glands, and tes es showed mas s e lymphoblast c inf ltrat on but there v ere no inf ltrates in the men nges

Comment

This young man had a lymphoblastic leukaemia Remiss on v as induced by prednisone but ten months later the leukaem a relapsed and shortly there after signs of superior mediastinal com press on developed At autopsy lympho-b ast c inf ltrat on of the liver kidneys testes and suprarenal glands v as dem onstrated Furthermore a thymic tumour probably a thymoma of lym pho d type v as found The numerous b g necroses in th s tumour ind cate its long durat on

Case 3

G rl born n 1955 Fam l and past his ory unremarkable In June 1966 anorex a and vom n set in X ray exam nat on of th chest showed enlargement of he superior n ed as num

The g rl was hosp talized Lymph nodes l ver and spleen were not enlarged The Hb concen rat on as 8 g 100 ml he leuko yte count 4 000/ l w th 12 % immature blast l ke cells h le the other cells were mature The thrombocyte count v as normal The bone marro smear sho ed immature cells v h ch ere d agnosed as myeloblasts They v ere characterized by ba ophilic cytopla m some mes conta n ng a fe granules and b large leptochromat c nuclei with prom nent nucleoli

The pat ent as transferred to the Ped at r c Department R gshospitalet She v as treated w th prednisone 6-mercap opur ne and blood transfus ons Later methotrexate was g ven also but no s gns of rem ss on ensued B lateral hydrothorax developed later pancytopen a and three months after the onset of symptoms the pat ent d ed

Autopsy

Emac ated g rl On the skin a great number of pe ech al bleedings was found

The l mph nodes were not enlarged In the med as num a gre h wh e rather sof un form tumour measuring app ox ma el 10 × 10 cm as demonstra ed n the reg on of the th mus It as poorly encap ula ed there as tumour nf l ra on do n a d n o he an er or wall of the per ard um and beh nd he heart n o he d aphragm he e rounded tumour nf l ra es mea ur ng up o 4 m n d ame er were found I a no pos ble to f nd ne ro e or cal f a ns macroscop call

Spleen l er and k dneys were normal



Fig 7 Case 3 Thymic tumour H E × 180

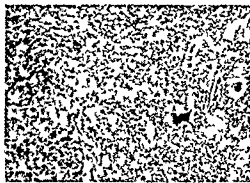


Fig 8 Case 3 Normal thymic tissue with Hassall's corpuscles right tumour tissue left H E × 73

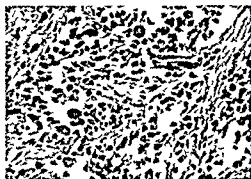


Fig 9 Case 3 Tumour tissue infiltrating the bone marrow (vertebra) H E × 180



Fig 10 Case 3 Vertebra with tumour infiltration and giant cells H E × 73

Microscopy

The mediastinal tumour was composed of rather small pleomorphic cells, arranged in a solid pattern Some of the cells had two nuclei The tumour was characterized by a well pronounced stroma with broad strands of fibrous tissue Scattered necroses were seen The tumour tissue was rather anaplastic, and it was impossible to decide whether the tumour was of epithelial origin (fig 7) In one of the slides normal thymic tissue with Hassall's corpuscles and calcification was found in intimate relation to the tumour tissue (fig 8)

The lymph nodes showed normal structure Plasma cells and blood pigment were abundant and the sinus reticulum was hyperplastic but no signs of metastasis or leukaemia were found

In bone marrow from the spine and the femur a tumour tissue closely corresponding to the thymic tumour was found everywhere (fig 9) Very large multinucleated giant cells were also found, they often contained phagocytized material in the cytoplasm and were possibly of foreign body type (fig 10)

There was no leukaemic infiltration in the liver spleen kidney, suprarenal glands or central nervous system

Comment

In this girl immature blast like cells were demonstrated in peripheral blood and bone marrow They were diagnosed as myeloblasts Chemotherapy did not induce remission, and the course was rapidly downhill Terminally bone

marrow failure developed. At autopsy an anaplastic tumour, probably originating from the thymus was found; the bone marrow was massively infiltrated with a morphologically identical tumour tissue.

Discussion

The analysis of 83 cases of thymic tumour and acute leukaemia by Cooke (5) established that this combination occurs far more frequently in males, and predominantly in the first three decades of life. The initial symptoms may be either leukaemic manifestations or mediastinal obstruction. The course of the leukaemia does not differ from that of cases with no thymic tumour.

From the clinical history, it is usually impossible to decide whether the thymic tumour or the leukaemia was primary. There are, however, cases on record in which a thymoma was found at a time when no leukaemic cells were seen even in bone marrow aspirates (2, 24); leukaemia became demonstrable weeks or months later. On the other hand cases have been published in which a thymoma was diagnosed only late in the course of the leukaemia.

Our Cases

The thymomas

The thymic tumours were classified according to morphological criteria (3, 14).

Case 1 had a lobulated thymic tumour which infiltrated the surrounding tissues; microscopically areas with lymphoid cells, spindle cells and rosette formation were found. Spindle cells and rosette formation are characteristic of thymomas and are never found in lymphosarcomas.

In case 2, the thymic tumour had invaded the greater part of the pericardium and was spreading around the mediastinal vessels. Yellow necroses measuring up to 3 cm in diameter were seen on the cut surface. The histological picture was predominantly lymphoid but here and there clusters of epithelial cells were found. This patient had massive leukaemic infiltration of the liver and kidneys. No necroses were found in these organs, and no therapeutic X-ray irradiation had been given to the mediastinum, so we do not regard the thymic necroses as a result of therapy. Castleman (3) mentions that necroses may be found in thymomas of long duration. In lymphosarcomas necroses are rare (7) and they are not at all mentioned by Lumb (18).

The thymic tumour of case 3 was composed of cells bigger than lymphocytes, arranged in a solid pattern with a well developed stroma. These tumour cells were anaplastic, possibly dedifferentiated epithelial cells.

In conclusion, the thymic tumours were probably neither lymphosarcomas nor leukaemic infiltrates but more likely genuine thymomas.

The leukaemias

In the first two cases the leukaemia was lymphoblastic and chemotherapy induced remission. In case 3, however, the leukaemia was subleukaemic; the malignant cells in the blood and bone marrow had the appearance of myeloblasts and a remission was not achieved.

The disappearance of the lymphoblasts

from the blood of case 1 after subtotal thymectomy, before any other therapy had been instituted, was striking It appears probable that the malignant cells in the blood originated from the thymoma

In case 2, the thymoma was diagnosed only late in the course of the leukaemia, but the necroses of the tumour indicated that it was of long standing It cannot, therefore, be excluded that the thymoma may have been the primary factor

In respect of the leukaemic state and of the thymic tumour case 3 differed significantly from the other two cases At autopsy, involvement of the thymus and the bone marrow was demonstrated but no further infiltration of the lymphoid organs This case has some resemblance to that published by Adams (1) although the tumour tissue in our case was much more dedifferentiated The massive infiltration of the bone marrow with anaplastic tumour tissue suggest that the malignant cells of the blood may have been anaplastic tumour cells although they have considerable resemblance to myeloblasts The terminal bone marrow failure was a natural consequence of the marrow infiltration besides, intensive chemotherapy had been given

Therapy

The treatment of concurrent acute leukaemia and thymoma is most often prednisone and/or a cytotoxic agent in some cases combined with high voltage X ray irradiation of the mediastinum, the thymomas are highly radio sensitive If, however, mediastinal obstruction is menacing operation (as in our case 1) is preferable

The hypothesis of a central role of the thymus in human acute leukaemia has led to the suggestion that thymectomy should be tried also in cases without enlargement of the thymus (25) The results of experimental work in mice do not lend support to this suggestion, since the frequency of leukaemia is reduced only by thymectomy early in life It is therefore not surprising that thymectomy in humans with acute leukaemia in whom remission was induced by cytotoxic therapy had little or no effect on the subsequent course of the disease (10)

Animal Studies

It was shown by Krebs et al (13) that radiation induced leukaemia in mice usually has its starting point in the thymus Cole and Furth (4) found that the thymus is the site of origin in spontaneous leukaemia in Ak mice McEndy et al (20) demonstrated that early thymectomy in mice of the same stock reduced the incidence of leukaemia considerably

Later work has confirmed the central role of the thymus in murine leukaemogenesis In several mouse strains it has been demonstrated that the first malignant cells occur in the thymus (11) In other strains with a similar high incidence of leukaemia and a similar protective effect of thymectomy morphologic thymic involvement occurs much more rarely however (16)

It is possible to re establish the usual incidence of leukaemia in thymectomized mice by thymus transplantation

the leukaemias then apparently originate in the thymic grafts (15) but only in strains with a high frequency of thymic involvement in spontaneous leukaemia (17) Re transplantation experiments (15) and chromosomal marker studies (22) suggest that the malignant lymphoblasts in most cases do not arise from the thymus transplant, but that thymic reticulum cells induce leukaemic transformation of the host s lymphocytes invading the transplant

How this more indirect effect of the thymus is accomplished is uncertain Several authors have described a lymphocytosis stimulating factor of thymic origin (21) A humoral mechanism has also been implicated by experiments in which the lymphatic tissues of neonatally thymectomized mice were at least partially repopulated after the implantation of thymus tissue in Millipore diffusion chambers impermeable to cells (23) It has, however, not been possible to restore the high incidence of leukaemia by this procedure

Connection Between Thymoma and Leukaemia

In rodents, it is established that the thymus plays a central role in a) lymphocytopoiesis, b) the development of immunocompetence and c) leukaemogenesis (8) In man, the immunological deficiency syndromes associated with thymic aplasia and lymphocytopenia suggest a similar importance of the thymus in lymphocyte production and immunological maturation (9) on the other hand, the association between human leukaemogenesis and the thymus is uncertain Our cases and those reported earlier do however show that in certain cases a connection exists between thymoma and leukaemia in man It is unknown whether the thymus is of any importance in that large majority of leukaemic patients in whom it is not grossly involved but this remains an attractive possibility deserving further study

Summary

Thymoma and coexistent acute leukaemia has been recorded so frequently in the clinical literature that a fortuitous coincidence is unlikely Three additional cases are now presented two with lymphoblastic leukaemia, while in the third the leukaemia was difficult to classify The experiments establishing the central role of the thymus in murine leukaemogenesis are reported and the possibility of a similar importance of the thymus in the development of human leukaemia is discussed

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Renal Biopsy in Rheumatoid Arthritis

By

A Pasternack O Wegelius and P Makisara

Kidney involvement in rheumatoid arthritis has attracted attention recently (5 10 16) It is still under debate whether the renal manifestations are to be interpreted as resulting from use of analgesics or whether there is a specific rheumatoid kidney disease In an attempt to contribute to the solution of this problem we performed renal biopsies on twenty patients with rheumatoid arthritis The following is a report of the results obtained

Material and methods

The patients were selected to represent both recent and long standing cases of rheumatoid arthritis In addition we studied ten consecutively selected control patients on whom the biopsy was performed only because of one or a few episodes of pyelonephritis All these control patients had normal renal function and none of them had used analgesics The main data of the patients are shown in table I

Group A is formed of ten patients with recent rheumatoid disease i e duration less than two years In four patients the duration was one year or less The patients in group A were selected on grounds of freedom from previous or present urinary tract infection Proteinuria or haematuria did not occur

Group B is composed of ten patients with long histories of rheumatoid arthritis In five patients the duration was more than ten years Five of the patients in this group were known to have had one or more episodes of pyelonephritis previously At the time of biopsy proteinuria haematuria and urinary infection were absent Four patients had an endogenous creatinine clearance of less than 80 ml/min/1 73 m^2 In one of these and in two other patients the renal concentrating capacity was subnormal as judged by a twelve hour dehydration test In six patients the phenol sulphonphthalein test (PSP test) was less than 55 %/2 hrs

Among the patients there was no history of heavy analgesic abuse Almost every patient had been treated with acetylsalicylic acid phenylbutazone and antimalarial drugs Ten patients had got moderate doses of corticosteroids In group A two patients had had no treatment with gold salts in seven patients the total dose (Myocrisin ®) was between 30—150 mg and in one pa

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TABLE I Main data of the patients

	Control group	Group A	Group B
Number of patients	10	10	10
Duration of disease yrs		2	2—30
Age of patients yrs	20—65 (40)[1]	16—59 (37)	21—65 (43)
Blood urea, mg%	14—39 (25 5)	18—48 (29 5)	13—39 (26 3)
Serum creatinine mg%	0 5—1 1 (0 82)	0 4—1 3 (0 74)	0 4—1 2 (0 73)
Endogenous creatinine clearance ml/min/1 73 m²	71—161 (95 1)	80—172 (115 6)	52—125 (83 5)
PSP test %/2 hrs	46—78 (65)	56—73 (57)	38—66 (51)
Urinary specific weight after 12 hrs dehydration	1 024—1 034	1 020—1 032	1 016—1 028

[1] Mean value

tient 400 mg In group B prolonged treatment with gold had been given in eight patients while two patients had not been treated with this agent

B P was normal in all patients

The renal biopsies were performed percutaneously using the Vim Silvermann needle The tissue was fixed in 10 % neutral formaldehyde embedded in paraffin wax and sectioned at 5 7 μ The sections were stained with haematoxylin van Gieson periodic acid Schiff and methyl violet for amyloid

TABLE II [illegible] found in renal biopsies [illegible] patients with rheuma[illegible] and ten control pa[illegible]

	Control group	Group A	Group B
Amyloidosis			1
Vascular changes	[illegible]	2	7
Local glomerulitis	2	6	5
Interstitial changes	1	3	6
No changes	8	7	—

Results

(Table II and figs 1—3)

Amyloidosis was seen in one of the cases belonging to group B The metachromatic material was concentrated in the glomerular tufts and vessels This patient was a male aged 50 years in whom the duration of the disease was over ten years

Interstitial tissue changes were seen in nine patients Six of them belonged to group B One of the control patients had this alteration The changes were characterized by an increase in collagenous tissue with only scanty round cell infiltration The increased interstitial tissue contained tubular portions with flattened atrophic epithelium and a PAS positive amorphous content This type of change was found in one patient 21 years of age with rheumatoid arthritis of two years duration, and in one patient 32 years of age with disease of ten years duration The seven additional patients with interstitial changes were all among the oldest patients in the

ser es The three patients with defect ve renal concentrat ng capac ty had th s type of les on

Glomerular changes appeared in six pat ents of group A and f e pat ents of group B The cellularity of the glomeruli as con idered normal Splitting of the basal membrane gave the impress on that t was th ckened Ho ever PAS sta n ng sho ed no membranous accumu lat on of PAS pos ti e material In local areas of the glomerul there was accumu lat on of PAS pos t ve material w th an ncreased number of nuclei in the same area The adjacent cap llary loops were v der than normal Two of the pat ents n the control group had th s type of les on

A ter olar thicken ng or *arterial th ck en ng* was seen in nine pat ents Two be longed to group A and n one of them female of 16 years no other changes ere observed In the seven pat ents of group B as in tw o control pat ents the vascular alterations occurred together th other changes

Discussion

That renal amylo dos is a common sequel to rheumato d arthritis is well kno n from numerous reports (5 8 9 12 13 17) A note orthy po nt is that amylo dos s may be present w thout ob vious renal d sease and w thout any cl n cal susp c on of amylo dos s This is sho n by the cases of Brun et al (5) Te lum and Lindahl (17) and our o n pat ent

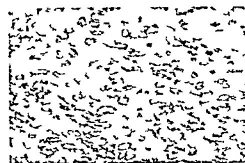
Chron c interst t al nephritis was a very common form of renal d sease in rheumato d arthrit s in the Dan sh series of Brun et al 5 and Clau en and Pe dersen 6 In contrast Pollak et al 13 d d n t f nd a s ngle case of inter stit al nephr t s in th e r b opsy material



F g 1 Local glomerul s Local a umula t on of nucle and many w de ap llary loops Pe od c ac d Sch ff × 500



F g 2 Per pheral part of glomerulus show ng local accumula on of nu le Pe od c ac d Sch ff × 1 000



F g 3 Renal medulla w h n reased amoun of n ers al connec e ue Some tubu a e seen w h amorphous plugs Haema toxyl n an G eson × 150

from the United States This fact parallels the relatively infrequent reports of interstitial nephritis from the United States as compared with certain European countries Analgesic abuse is the aetiological factor most commonly incriminated when non pyelonephritic interstitial nephritis is concerned (3, 11) Many patients with rheumatoid arthritis consume remarkable amounts of analgesics including phenacetin and salicylates The older the patient the greater the possibility of heavy analgesic abuse Most of our patients with this type of renal disease were old and had prolonged duration of the rheumatic disease thus the possibility exists that analgesics were the cause On the other hand we excluded heavy analgesic abuse from the series and found interstitial changes in one patient of only 21 years of age Therefore we cannot state that analgesics are the only aetiological factors causing renal interstitial changes in rheumatoid arthritis although they may be the most important It is to be noted that we found interstitial changes in only one of our control patients

Diffuse glomerulonephritis as described in a few authors (1 13 14) was not found in our series The glomerular alterations in our series correspond to those described by Brun et al (5) namely some kind of local glomerulitis This type of glomerular change has also been observed in some renal biopsies from normal individuals (4) In our series two of the controls had this alteration In four patients the local glomerulitis was found to be the only renal abnormality, in seven patients it was followed by either interstitial and or vascular changes When we add to this the splitting of the basal membranes and the fact that the changes were seen in several glomeruli in the same biopsy we feel that we might be dealing with an early pathological alteration of rheumatoid kidney disease although we do not believe it is specific The role of drugs in producing these changes is extremely difficult to evaluate In the group of early rheumatoid arthritis however, the local glomerulitis was found without any relation to drug therapy

No necrotizing arteritis was observed in this series, in contrast to previous reports (2, 7, 15), possibly owing to the exclusion of cases with clinical kidney disease Arterial and arteriolar sclerosis was found mainly in the old patients or in young patients in connexion with other changes The vascular changes are probably a manifestation of the ageing of the kidney The frequency of vascular alterations in the patients with long standing rheumatoid arthritis gives the impression that this might be an essential component of the more advanced rheumatoid kidney The thickening of the arterioles in one of our young patients with recent rheumatoid arthritis remains obscure

Summary and conclusion

Renal biopsies were studied in twenty patients with rheumatoid arthritis and ten control patients The patients had both recent and long standing rheumatoid arthritis and only cases free from obvious renal disease and from analgesic

abuse were selected Amyloidosis was found in one patient In nine patients arterial or arteriolar sclerosis was found probably related to both ageing of the kidney and advancement of the rheumatoid disease The presence of so called *local glomerulitis and interstitial tissue* changes in young patients with recent onset of rheumatoid arthritis and no heavy analgesic abuse makes the existence of a rheumatoid kidney disease at least probable

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Observations on the Diagnostic and Prognostic Value of Some Enzyme Tests in Myocardial Infarction

By

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Since 1954 when LaDue et al (17) first measured the activity of serum glutamic oxalacetic transaminase in myocardial infarction this and other enzymes have been widely used as diagnostic aids

Their value has proved to be high, but although much work has been done in this field (see e g reviews by Agress (1, 3) and Wroblewski (28)) there still remains some uncertainty as to the exact degree of their reliability and to the comparative usefulness of the various enzymes

The object of the present study was to analyse the diagnostic validity of some enzymatic methods in patients with myocardial infarction in whom this diagnosis had been established by other methods, and to examine the relative diagnostic reliability of the clinical and electrocardiographic methods In addition, an attempt was made to evaluate the prognostic significance of the enzymatic data

Submitted for publication April 18 1967

Material and methods

Our case material consisted of patients admitted to the Department of Medicine Serafimerlasarettet Stockholm between January 1 1956 and June 30 1959 suffering from myocardial infarction verified by means of one or several of the following criteria

1) the infarction was demonstrated at autopsy or
2) the clinical picture was typical or
3) the electrocardiogram was typical or
4) both the clinical picture and the ECG were judged as probable infarction

All patients were studied by means of ECG and enzyme tests as specified below The material consists of 160 infarction in 155 patients Of this number 58 patients (59 infarctions) were examined post mortem The majority of the post mortem examinations were performed at the Department of Pathology Serafimerlasarettet under the direction of Dr H Nordenstam Special regard was paid to analysis of the cardiac lesions

In evaluating the clinical diagnosis we have followed the principles employed by Forssman (8) Chest pain was considered obligatory for a positive clinical diagnosis it was absent in only two cases which

TABLE I Point scale for evaluation of clinical signs

Points	Max leuk value first 4 days (count/mm³)	Mean morning rectal temp first 4 days (° C)	Rise in ESR first week (mm/hr)
0	< 9 000	<37 1	<20
1	9 000—11 900	37 1—37 5	20—39
2	12 000—14 900	37 6—38 0	40—59
3	>14 900	>38 0	>59

were classified as negative In addition, a reaction from the leukocytes the temperature and the ESR was required according to table I

Three points—based on at least two of the three sources of information in the table—were demanded for a definite diagnosis and two points for a probable diagnosis If less than two points or two from one source only were obtained the diagnosis was considered negative In about one third of the clinical diagnoses the symptoms included varying degree of shock Hyperglycaemia was a common finding However in view of the high incidence of manifest and latent diabetes in the material our observations with respect to the blood sugar level were disregarded

ECGs were recorded with a high fidelity direct writing 4 channel electrocardiograph (Mingograf Elema) The following 16 leads were used I—III aVL, aVR aVF $CR_{1-4,5,7}$ and $V_{1,2,4,7}$ (in single instances the V leads and CR, were omitted) As a routine ECGs were recorded daily for the first few days after which the interval was increased to a week In the evaluation all ECGs taken within the first 30 days were considered

They were evaluated according to generally adopted conceptions (cf 27) consideration being paid however to the fact that the problem was not limited to the question whether signs of cardiac infarction were present but also included the decision whether a fresh infarction was developing during the observation time A considerable proportion of the patients had had infarctions previously Among those examined post mortem in whom this fact could be observed with the greatest certainty, the incidence was about 38 % Therefore, obvious infarct signs had often to be disregarded as in the case of old ECGs showing a previous infarct and also when an atypical ECG picture was stationary during a sufficiently long observation or did not change in a manner compatible with the evolution of a fresh infarction Generally great importance was attached to the time course of the changes

In the final ECG diagnosis, therefore, the different Q S—T and T items were normally evaluated with regard to their compatibility with the evolution of a fresh infarction In the ensuing pronouncement of a positive , probable or negative electrocardiographic infarction diagnosis it has not been possible to avoid a certain degree of subjectivity, since an account of the observed signs in the manner of e g the Minnesota Code (5) would not be truly objective because as described above identical signs have had to be interpreted differently in different cases In certain cases static ECG pictures have had to be appraised This is especially true of the 4 instances of early death of the patient, when only one ECG had been made Three of them came to autopsy fresh infarctions were demonstrated in all three as well as old infarctions in two In one of these two the ECG diagnosis was that of a probable infarction based on elevated S—T segments and intraventricular conduction de

fect but no Q in the other three single ECG cases it was positive with large pathologic Q and negative T waves as well as elevated S—T segments and rhythm disturbances Of the patients who were not subjected to autopsy 61 fulfilled both the second and the third criterion with regard to verification of infarction (see above) 16 the second only, 16 the third only and 8 the fourth only

Patients were included in the series only when at least two determinations of glutamic oxalacetic transaminase (GOT) and two determinations of lactic dehydrogenase (LD) had been made within seven days of the anamnestic episode taken as indicating the onset of infarction Exceptions to this rule were made in five cases early in the series when LD determinations were not yet part of the laboratory routine, as well as in the five patients who died so soon after admission that time permitted only one enzyme determination The analyses were performed at the hospital's enzyme laboratory under the direction of Dr R Ordell by his modification (21) of the method introduced by Karmen and Wróblewski (12 30) In this modification the numerical values are usually about one tenth of those according to the original method The normal values as given by Ordell (21 22) are 8—30 units for both GOT and LD We have judged values in the region of 31—39 as probably and those over 39 as definitely pathological During the latter half of the observation period glutamic pyruvic transaminase (GPT) was also determined in each blood specimen using Ordell's modification of the Wroblewski method (29), with a normal range of 12—30 units (22) The number of GPT analyses is therefore comparatively small in this study they were used principally as an aid in the recognition of complications—mainly liver damage No detailed analysis could be made of their relation to the cardiac infarctions as such

The total number of admissions for the diagnosis of myocardial infarction in the period in question amounted to 269 Altogether 109 had to be excluded from the present study 52 because no enzyme test was made 24 because enzyme tests could not be performed until more than seven days had elapsed from the probable onset of infarction six who at the time of infarction had other diseases which obscured the initial evaluation of the clinical symptoms and signs nine with insufficient data (e g time of enzyme determination not mentioned no ECG taken patient demanding discharge from hospital within the first 2—3 days) and 18 cases which for other reasons did not fulfil our aforementioned diagnostic criteria

Of the remaining 160 infarctions 121 (76 %) were in males and 39 (24 %) in females the male/female ratio is thus 3 1 1 The average age was 64 years (males 62, females 68) The youngest patient was 33 years old the oldest 87 The six week mortality rate was 43/160 = 27 % (males 31/121 = 26 % females 12/39 = 31 %) The average age of those who died within six weeks was 68 years (males 65 females 74)

In these respects the total group of 269 cases is almost identical with the group comprising the present series

When two infarctions in the same patient were included in the series they had caused hospitalisation on two separate occasions The shortest interval between them was three months Infarct extensions in the course of a single hospitalisation occurred in 13 cases they were not regarded as separate infarctions Here the longest interval was four weeks

Results

Autopsy cases

When evaluating the results of enzyme tests in myocardial infarction special attention must be paid to those cases in which autopsy has been performed It is true that many observations of this

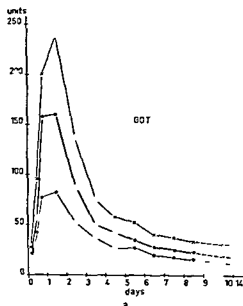
TABLE II Autopsy cases Number of diagnoses by different methods in 59 infarctions

Diagnostic method	Diagnosis					
	Positive		Probable		Negative	
	(No)	(%)	(No)	(%)	(No)	(%)
ECG	42	71	8	14	9	15
Clinical	44	75	10	17	5	8
Comb ECG+clin	51	86	4	7	4	7
Enzymatic	58	98			1	
GOT alone	58				1	
LD alone	53				2	

kind have been published, several well documented Scandinavian reports being among the largest autopsy series (4 10 11, 19) Nevertheless according to the reviews by Agress (1, 3), the total number of infarctions 'with autopsy proof amounts to only 119 For this reason a special analysis has been made of the 59 infarctions (58 patients) in the present series in which an autopsy was performed The relative validity of the different diagnostic methods is presented in table II

Judged by our clinical diagnostic criteria five of the 59 cases were negative Of these five three had a positive ECG one a probable and one a negative ECG Of the nine ECG negative cases six had changes produced by earlier infarctions which obscured the result one had a left bundle branch block and one was demonstrated at autopsy to have diffuse myomalacia rather than a definite infarction Diagnosed clinically, six of them were positive two probable and one negative The combined diagnosis (i e clinical + ECG) was negative in four cases Of these four ECG tests gave negative findings in three and a probable finding in one, whereas the clinical diagnosis showed two negative and two probable Thus, in one case the results were negative, according to the aforementioned diagnostic criteria on both clinical and electrocardiographic grounds The ECG was obscured by at least one earlier infarction There was, however, a history of retrosternal pain two days before admission, a fall in systolic blood pressure from 140 to 105 mm Hg and blood sugar 0 15 % on admission falling in two days to 0 11 % No fever, leukocytosis or rise in sedimentation rate occurred The patient died after four months The enzyme tests were positive, the values on the 2nd 3rd and 9th day being 90—90—12 units for GOT 34—47—22 for LD and 16—22—18 units for GPT

As for the *enzyme tests* the reaction in GOT and LD activity produced by the onset of myocardial infarction in our series is illustrated in fig 1 The curves show a general agreement with similar values previously reported (15, 18 23—26)



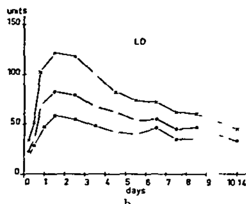


Fig 1 Median and quartile values for the maxima of GOT (a) and LD (b) in the course of the cardiac infarctions in this study

As is seen from table II only one of the 59 cases is negative in the combined evaluation with GOT and LD This case was positive from the clinical and electrocardiographic points of view The patient was a man of 52, he was admitted to the hospital one afternoon with typical chest pain which had begun at 10 a m ECGs were taken at 3 and 5 p m and a blood specimen for enzyme analysis at 9 on the following morning i e in the 23rd hour The patient died one hour later and at autopsy was found to have a rupture of a medium sized fresh posterior infarction His enzyme values were GOT 18 LD 30 and GPT 12 units The ESR determined concurrently was 17 mm/hr Although only one enzyme analysis was performed it was made at a time—23 hours after the onset of symptoms—when it should have shown definitely elevated values at least for GOT We have been unable to find any convincing explanation of the negative results in this case

In all the remaining 58 infarctions GOT showed pathologically high values LD was determined in 55 cases and found to be pathological in 53 One of the two negative cases has just been mentioned The other patient also died so soon that only one enzyme determination could be made This showed GOT 64 LD 28 and GPT 20 units The blood specimen was however taken only 15 hours after the onset of symptoms when as seen in fig 1 b almost one fourth of all cases still have normal values It is therefore not improbable that the LD activity in this case would have become pathological within the next few hours and that this value therefore constitutes a false negative result Thus with one exception (possibly two in the case of LD) the infarctions

TAB III Non autopsied cases Number of diagnoses by different methods in 101 infarctions

Diagnostic method	Diagnosis					
	Positive		Probable		Negative	
	(No)	(%)	(No)	(%)	(No)	(%)
ECG	77	76	12	12	12	12
Clinical	77	76	12	12	12	12
Comb ECG+clin	93	92	8	8	—	—
Enzymatic	99	98	1	1	1	1
GOT alone	94		5		2	
LD alone	90		6		4	

verified by autopsy were diagnosed by determination of each of the two enzymes in every case in which this was done within the appropriate time limits

Non autopsied cases

An analysis of the non autopsied cases —101 infarctions in 97 patients—is given in table III The proportion of negative clinical and electrocardiographic examinations was similar to that in the autopsy series (table II) The combined clinical and electrocardiographic evaluation could not be negative in any of these case as the series was selected precisely on this basis

The only enzyme negative case in this series was negative clinically as well, but had a typical ECG The patient was a 67 year old woman with acute retrosternal pain starting 3—4 hours before admission She was somewhat cyanotic but had no fall in B P no leukocytosis nor any rise in temperature or ESR The ECGs showed an unquestionable fresh anterior infarction Her enzyme values 4, 24 and 48 hours, respectively, after the onset of symptoms were GOT 18—29—25 and LD 20—21—29 units The corresponding values 15 and 16 days after the infarction were 23—20 and 19—20 units

As far as the relative role of the two enzymes is concerned, GOT determinations were made in all 101 cases, and showed elevated maximum values in all but two One of these has just been discussed The second showed GOT falling in three days from 21 to 13 units, and LD from 38 to 14, which is compatible with an infarction five or six days old This also applied to the ECG, which showed QS in the first tracing According to the history however the patient was hospitalized on the day of onset

LD was determined in 100 cases and showed elevated values in 96 One of the four cases in which LD determinations failed to diagnose infarction has already been mentioned The other three were all ECG positive but showed only slight clinical signs, the clinical diagnosis was considered probable in one case and negative in two Their GOT maxima, on the first or second day, were 68 35 and

TABLE IV All cases Number of diagnoses by different methods in 160 infarctions

Diagnostic method	Diagnosis					
	Positive		Probable		Negative	
	(No)	(%)	(No)	(%)	(No)	(%)
ECG	118	74	20	12	22	14
Clinical	121	76	22	14	17	11
Comb ECG+clin	144	90	12	8	4	2
Enzymatic	157	98	1	1	2	1
GOT alone	152		5		3	
LD alone	143		6		6	

45 units respectively the LD maxima on the 2nd to 4th day were 29 15 and 22 units

A *survey of the diagnostic validity in* the whole series of 160 infarctions is given in table IV

Size of infarct

A correlation between the elevation of enzyme activity in the serum and the size of the infarct has been demonstrated in several experimental studies (2 20 23) This correlation in our series is demonstrated in tables V (GOT) and VI (LD) Here all infarcts are included in which there were satisfactory autopsy reports on the dimensions and the highest enzyme value was obtained within 1—3 days of the onset in the case of GOT *and 2—4 days in the case of LD*

The correlation could be analysed in 49 infarcts with respect to GOT and 50 with respect to LD The infarcts were graded as small when the dimensions were given as coin size smaller than the palm of a child s hand or under 20 cm² medium sized up to an adult palm or 60 cm² and large over this size The correlation must be regarded as good *especially if it is borne in mind that the* estimation of infarct size however conscientiously made must remain fairly rough and that the highest enzyme value recorded is not necessarily close to the real maximum

TABLE V Relation between infarct size and GOT maximum

GOT max units	Infarct size		
	Small	Medium	Large
> 499			4
300—499	1	3	6
200—299		5	3
100 199	9	8	1
50— 99	6	2	
< 50	1		
GOT median	108	178	435
No	17	18	14

TABLE VI Relation between infarct size and LD maximum

LD max units	Infarct size		
	Small	Medium	Large
>249			4
200—249		1	2
150—199	1	4	4
100—149	6	7	1
50— 99	10	8	1
< 50	1		
LD median	84	109	193
No	18	20	12

Immediate mortality

As demonstrated in fig 2, the corresponding correlation to the immediate mortality rate is also present in the series, this applies to both GOT and LD

Thus, 49 % of the patients who had a GOT maximum over 250 units died within six weeks, whereas only 20 % of those under 250 units died in the same period This is in general agreement with the findings of other authors (6, 7, 10, 13—16, 25) The corresponding figures for LD are 64 % of all patients with a maximum over 150 units, as compared to 21 % of those with a lower maximum

Late mortality

The possible significance of the enzyme maximum for the long term prognosis of patients surviving the acute stage of the infarction has not, to our knowledge, been investigated previously

In the present investigation, 117 infarction cases survived six weeks We have collected information concerning all these patients after an observation

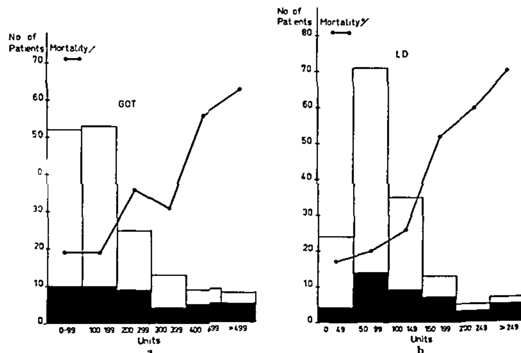


Fig 2 Six week mortality rate in relation to maximum value for GOT (a) and LD (b)

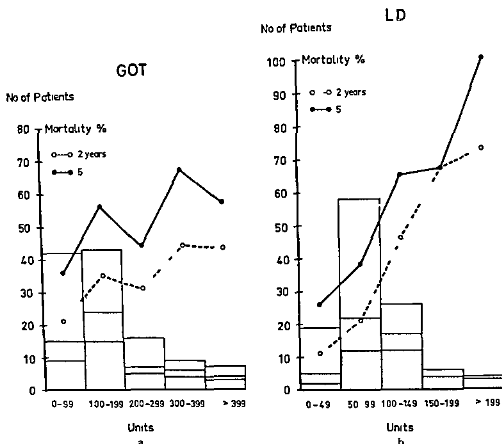


Fig 3 Two nd five year mortality rates in relation to maximum value for GOT (a) and LD (b)

period of five years and analysed their mortality after two and five years they are accounted for in fig 3 In four of the cases no LD determination was per formed fig 3 b therefore represents only 113 cases

It is seen that the two years mortality rate increases with rising GOT and LD maxima This impression is confirmed by a χ test which demonstrates the cor relation to be significant on the 5 % level for both enzymes The actual rate of increase in the case of GOT is, how ever small so that the mortality at val ues over 300 units is only about twice that in the region 0—99 In the case of LD in contrast, the difference in mor tality rate is large the values in the highest LD range being more than six times those in the lowest range Ex tremely low GOT values were recorded only in survivors The lowest value in any patient who died within two years was 58 units For LD this tendency is not pronounced the lowest values in non surviving patients being 35 and 46 units (combined with GOT 110 and 102 units)

mands a comparatively large number of analyses, a circumstance that must be weighed against the greater diagnostic reliability. The degree of the latter in our series is evident from the fact that one more of the 160 cases would have been missed if GOT alone had been used and that four of the cases now regarded as definitely diagnosed would have been classified as probable. Exclusive use of LD would have yielded an additional number of four negative and five probable cases. Thus, judging by this study, GOT appears to be somewhat superior to LD in the diagnosis of myocardial infarction. Our general conclusion therefore, in agreement with Hanson and Biorck (10), is that GOT alone suffices in most cases but should be supplemented by LD especially when several days have elapsed between the infarction and examination of the patient. In this way some simplification of the procedure might be accomplished at only insignificant cost in diagnostic accuracy. It must nevertheless be realized that the initial decision as to which enzyme tests are to be performed is critical in so far as an omission at the time of the first examination cannot in most cases be rectified later.

Our material shows a correlation between maximal enzyme values and mortality rate not only for the time immediately after the infarction but also for the following five years. Although in most respects the two enzymes react very similarly, in this case, for the two- and five-year periods LD seems to be a more sensitive indicator of the mortality rate than GOT. The comparatively small number of cases, especially in the ranges with high enzyme values, however, makes further analysis of this problem desirable.

Summary

The serum activity of glutamic oxalacetic transaminase (GOT) and lactic dehydrogenase (LD) has been serially determined in 160 cases of myocardial infarction (155 patients) in the period January 1, 1956—June 30, 1959. Clinical and electrocardiographic examinations were evaluated according to stringent principles to ensure that the series consisted only of definite infarctions.

Totally 58 patients—representing 59 infarctions—were examined at autopsy. Of these electrocardiographic examination had failed to diagnose nine (generally because of older, obscuring lesions), clinical evaluation five, and enzyme analysis one (determination of both enzymes). The combination of clinical and electrocardiographic examinations failed to diagnose four infarctions.

Of the 101 infarctions not verified at autopsy, GOT determinations diagnosed all but two, LD all but four and GOT and LD combined all but one.

A correlation is demonstrated between the size of the infarct and the maximum level of each enzyme.

A similar correlation is shown between the mortality rate within the first six weeks and the enzyme maxima.

Altogether 117 infarction cases discharged alive from hospital have been observed for five years. Their mortality rate after two and after five years are found to rise significantly with the peak

values for each enzyme The increase in mortality rate is moderate for GOT steeper in the case of LD In this series there is no correlation between the age of the patient and the maximum value of either enzyme

It is concluded that combined serial determination of GOT and LD practically never fails to establish the presence of a myocardial infarction, and that it is superior even to the combined clinical and electrocardiographic examinations The maximum value for each of the enzymes gives an indication of the size of the infarct as well as of the immediate and long term prognoses

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Resuscitation in Cardiac Arrest

An analysis of 100 successive medical cases

By

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The resuscitation treatment of medical cases was made possible by the establishment of an effective external cardiac massage technique by Kouwenhoven et al (11) and the introduction of direct-current defibrillators in 1962 (12)

Material and method

The series comprised 100 successive cases of cardiac arrest which were treated in the medical intensive care unit (ICU) of Tampere Central Hospital from March 1965 to September 1966 The youngest patient was 38 and the oldest 81 year old Sixty-eight were men, and thirty two women The diagnoses are shown in table I The majority suffered from acute myocardial infarction These patients were divided into three groups according to the severity of infarction employing the coronary prognostic index (CPI) proposed by Peel et al (18)

Messer (15) has recently reviewed in detail the procedures for treating cardiac arrest Our methods have been similar Some unusual features may be mentioned We have not appointed a special resuscitation team because it could not always be summoned in time Every physician in the medical wards was trained in the resuscitative measures and thorough training also given to the nursing

Table I Main diagnoses of the resuscitated patients

	No of pats
Acute myocardial infarction	63
Miscellaneous medical cases	37
Atherosclerotic hypertensive and valvular heart disease[1]	19
Aortic disease (ruptured dissecting aneurysm or coarctation)	4
Cerebral vascular disease	4
Pulmonary embolism	4
Diverse (drowning electrocution thyroid crisis poisoning)	6

[1] In two cases cardiac arrest followed attempts to effect conversion with quinidine and in one case D C countershock

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staff of ICU and to the staff of general medical wards and emergency room.

In all cases of cardiac arrest, external cardiac massage was initiated without delay and the patient monitored as soon as possible. For the reversion of ventricular fibrillation direct current countershocks (100—300 ws) were discharged with precordial or antero-posterior electrodes. Pure oxygen was administered using an intermittent positive pressure respirator (Bennett type PR 1 or PR 2) and a mask and a thick pharyngeal tube. Intubation was performed only when the treatment was prolonged. It was avoided initially in order to save time and because intubation often induces detrimental vagal reflexes (25).

Sodium bicarbonate (7.5 %) was infused as early as possible. Initially 100—200 ml (90—180 mEq) was given and an additional 100 ml was infused every 10—15 minutes during prolonged resuscitation. The amounts were somewhat larger than those generally recommended (4, 8, 24). If ventricular defibrillation was not successful after metabolic acidosis had been corrected or if the fibrillation recurred, propranolol (Inderal® ICI) was administered intravenously. It was also given after successful defibrillation to remove other arrhythmias and sometimes to maintain the corrected rhythm, although in most cases oral quinidine (Kinidin duretter® Hassle) was administered for the latter purpose.

Effective external cardiac massage reverted asystole in many cases, especially when D.C. countershock had converted ventricular fibrillation to asystole. Other measures included use of intravenous 0.01 % epinephrine and 10 % calcium chloride together with the infusion of metaraminol and norepinephrine which were administered to combat hypotension. The use of an external pacemaker was abandoned as it proved ineffective.

Particular attention was directed to careful monitoring for 5—10 days after the resuscitation. The patients were then tranferred from ICU to the adjoining ward from which they could be rapidly removed to ICU if needed.

Results

Resuscitation was attempted in 132 episodes of cardiac arrest in 100 patients. The treatment was successful in 73 episodes. Twenty seven patients were discharged alive after they had recovered from one or several episodes. (Cardiac arrest is in this connection defined as a state characterized by the cessation of effective circulation, due to ventricular fibrillation or standstill. A resuscitation episode was taken to be a separate one if more than one hour had elapsed from the former arrest. A resuscitation attempt was considered successful if satisfactory heart function was restored for one hour or more and spontaneous respiration returned.)

Age and sex

The distribution of cases into two age groups and the number of survivors in both groups are shown in table II. Thirteen (30 %) of the patients aged below 60, and fourteen (25 %) of the subjects aged 60 or more survived and left hospital. Nineteen (28 %) of the men and eight (25 %) of the women were discharged alive (table III).

TABLE II. Results of resuscitation in different age groups

Age (yrs)	No of pats	Unsuccessful	Temporary recovery	Left hospital alive
30—59	43	22	8 (19 %)	13 (30 %)
60—89	57	29	14 25(%)	14 (25 %)
Total	100	51	22	27

TABLE III Results of treatment in male and female patients with myocardial infarction and other medical diseases

	No of pats	Recent cases of myocardial infarction		Miscellaneous cases	
		Total no	Left hospital	Total no	Left hospital
Men	68	52	16	16	3
Women	32	11	3	21	5

TABLE IV The nature of rhythm disturbances in 132 episodes of cardiac arrest

	No of episodes	Unsuccessful attempts	Successful attempts
Ventricular fibrillation	77	22	55 (71%)
Asystole	38	28	10 (26%)
Undefined rhythm disturbance (no ECG could be recorded)	17	9	8 (47%)

Type of rhythm disturbance

Ventricular fibrillation was confirmed to be the cause of cardiac arrest in 77 episodes In 55 of them the resuscitation attempt was successful (table IV) The number of countershocks given per episode is shown in table V In four episodes ventricular fibrillation was abolished with external cardiac massage alone Their obvious cause was a quinidine syncope During one episode 43 defibrillations were required and yet the patient survived and left hospital Defibrillation resulted in a prolonged asystole during 29 episodes in 23 patients In 24 episodes asystole was reverted to ventricular fibrillation or other rhythm by cardiac massage Fourteen such episodes were treated successfully and eight of these patients were discharged alive

Asystole was verified to be the primary condition in 38 episodes of cardiac arrest Ten were successfully treated (table IV) The initial result was often good with no drugs except sodium bicarbonate

TABLE V Number of direct current counter shocks given in episodes of ventricular fibrillation

No of defibrillations	No of episodes	Unsuccessful attempts	Successful attempts
0	5	1	4
1	24	6	18
2—5	36	9	27
6—20	11	6	5
21—	1	0	1
Total	77	22	55

Nevertheless only one of the patients was discharged alive

In 17 episodes the type of cardiac arrest remained obscure because there was no time for monitoring Eight of the episodes were successfully treated by external cardiac massage and other measures (table IV)

Places where cardiac arrest occurred

Seventy nine episodes of cardiac arrest occurred in the ICU (table VI) Half

TABLE IX Comparative results of cardiac resuscitation in different series

Investigators		No of pats	Left hospital	Cases of acute myocardial infarction	
				No of pats	Left hospital
Himmelhoch et al	1964	65	4 (6%)	22	1 (5%)
Kaplan and Knott	1964	100	6 (6%)	—	—
Day	1965	—	—	16	9 (56%)
Nachlas and Miller	1965	—	—	60	3 (6%)
Pedersen et al	1965	177	25 (14%)	40	0
Robinson et al	1965	—	—	38	8 (21%)
Smith and Anthonisen	1965	128	23 (18%)	38	7 (18%)
Stemmler	1965	103	5 (5%)	26	1 (4%)
Goble et al	1966	—	—	28	7 (25%)
Grace and Minogue	1966	—	—	108	15 (14%)
Hansen and Sandoe	1966	47	16 (34%)	28	9 (34%)
Medford	1966	56	10 (18%)	28	6 (21%)
This study	1966	100	27 (27%)	63	19 (30%)

had atrial fibrillation and one had a regular heart rate maintained by an implanted pacemaker

In none of the re-examined patients had the cardiac arrest led to the development of neurological disturbances The memory was, however slightly impaired subjectively in seven patients

Discussion

The results of cardiac resuscitation reported from different centres vary due to differences in the series of patients and some consider that a comparison of the results is not justified (4) The chance of success is greater in surgical cases because the conditions causing cardiac arrest (hypoxia hypercapnia increased vagal stimulation) are usually more readily reversible in these than in medical cases (10) Table IX describes reported results, mainly in medical cases Better results can of course be obtained with selected series than with unselected successive cases

Opinions vary about the relative merits of internal and external cardiac massage Harley's (8) view is that the chance of success decreases rapidly if external cardiac massage does not restore adequate heart function within ten minutes After his paper was published a number of reports appeared describing patients who had been successfully resuscitated although external cardiac massage had been continued for a long period (1, 2) In the present series cardiac massage was continued more than fifteen minutes in 53 episodes (40 %) but despite this the attempt was successful in 16 episodes (30 %)

It has not been considered possible to use a pressure-cycled respirator for artificial ventilation during external cardiac massage (15) We have used such an apparatus (Bennett PR 1 and PR 2)

during external cardiac massage with good results Stock (25) and Nachlas and Miller (16) do not recommend intubation in the early stage of resuscitation, and we agree with them According to Stock intubation may induce ventricular fibrillation when the patient is hypoxaemic Our experience as well as that of many others has shown that an external pacemaker is ineffective in the treatment of asystole (5, 9 10 15), whereas Day (3) states that an automatic external pacemaker is of significant value

The administration of sodium bicarbonate has been found to improve the effectiveness of cardiac resuscitation by combating metabolic acidosis (e g 22) Our results confirm this opinion However, Himmelhoch et al did not find any beneficial effect on administering sodium bicarbonate

For the treatment of recurrent ventricular fibrillation Goble et al (5) used propranolol and procainamide Stock (25) recommended propranolol or procainamide after unsuccessful direct-current countershock before further electric shocks were resumed We have also used propranolol for both purposes and believe that it is of value

Information about the rhythm at the time of cardiac arrest is conflicting In two series (10 23) ventricular asystole was more common than ventricular fibrillation the latter was more frequent in patients with myocardial infarction in other series (10 16 19) The prognosis varies in both types of cardiac arrest The best results were obtained when patients with ventricular asystole were resuscitated (10) whereas Goble et al (5) reported that the prognosis is much less favourable in cases of ventricular asystole There was almost total failure with secondary asystole (14)

Table IV shows that ventricular fibrillation was more common than asystole in our series and also the prognosis was better Only one of the patients with asystole was discharged from hospital Recurrent cardiac arrest has led to progressively less favourable results (4) but our results reveal that recurrent cardiac arrest does not necessarily imply a poorer prognosis

Age has not been found to influence the results of resuscitation (4 25) and our findings confirm this

The place where cardiac arrest occurs might be expected to influence the prognosis of resuscitation Very serious medical cases which were considered in need of special care and supervision were admitted to the intensive care unit This may explain why only half of the cardiac arrests that occurred there responded to resuscitative procedures (table VI) Patients who had been successfully resuscitated in the intensive care unit were transferred to adjoining ward after a period of 1—2 weeks In our experience the chance of successful resuscitation when such a patient suffers a recurrence is relatively high Cardiac arrests that occurred in other medical wards were unexpected and the conditions of the patients were generally good before the attack This may account for the success of the resuscitative measures in over two thirds of the episodes in these patients

When the results of resuscitation in our series are compared with results in other series (table IX) we find that

they are favourable In many series more patients were initially resuscitated than in our series, but the proportion who survived and left hospital was higher in our series This applied to patients suffering from myocardial infarction as well as to the whole series An effective circulation and spontaneous respiration followed resuscitation in 42 % of the patients of Stock s series (25) and 13 % survived Spontaneous heart action was restored to 83 (47 %) of the 177 patients in the series of Pedersen et al (17) and 25 (14 %) survived Twenty nine of the 100 patients in Kaplan and Knott s series were successfully resuscitated, but only six were discharged from the hospital (10)

In our series comprising 132 episodes of cardiac arrest resuscitative procedures were initially successful in 73 episodes (55 %) Twenty seven per cent of the patients left hospital

According to Stemmler (23), the difficulty was to maintain effective rhythm in patients with myocardial infarction, rather than to restore it A satisfactory rhythm was restored in 12 (46 %) of the 26 patients of his series, but only one patient lived long enough to leave the hospital In the series of Nachlas and Miller (16) and Robinson et al (19) resuscitation was successful in 78 and 54 %, respectively, of the patients with myocardial infarction but only 5 and 21 % of the patients were discharged from the hospital alive In a survey of previous series, it is concluded that heart action can be restored in a relatively high proportion (32—70 %) of patients, but the proportion who survive is much lower (5—15 %) (16) In our series an effective rhythm was restored in 30 (48 %) out of 63 patients with myocardial infarction and 19 (30 %) of them were discharged alive

In cases of myocardial infarction, the first five days in hospital were observed to represent a high risk period and 83 % of the patients sustained cardiac arrest during this time (16) In our series, 63 % of cardiac arrests occurred within the first five days in hospital The fact that over one third of the episodes occurred later suggests that the monitoring and supervision of patients with myocardial infarction should be continued for a sufficiently long period in the intensive care unit

Shillingford (20) considered that more studies and larger series are required before it can be decided whether it is advantageous to establish intensive care units for specialized treatment of patients with myocardial infarction in hospitals We hope that our paper will contribute to these studies We are convinced of the value of special units in the treatment of myocardial infarction This conclusion is supported also by the results of other recent investigations (e g 6)

Conclusions

The results of this study clearly support the opinions that are beginning to prevail on the prerequisites and principles of cardiac resuscitation

By suitable training the nursing staff should be prepared to initiate resuscitative measures immediately a cardiac arrest occurs, regardless of the ward where the patient is located

A patient with cardiac arrest should be transferred as soon as possible to an intensive care unit for treatment and continuous supervision by trained medical and nursing staff The resuscitative measures should be continued effectively while the patient is being moved, for example, by using equipment installed on a movable bed Continued personal supervision after the resuscitation makes it possible to resume effective resuscitative measures when this proves necessary

Recurrent cardiac arrests can be treated as successfully as the first episode If ventricular defibrillation leads to asystole effective resuscitative measures should be continued since asystole of this kind does not necessarily impair the prognosis

Prophylactic administration of antiarrhythmic drugs during resuscitation and for a period of at least two weeks afterwards prevents the recurrence of lethal rhythm disturbances A condition for the success of electrical defibrillation and maintenance of adequate rhythm is the correction of metabolic acidosis by administration of sodium bicarbonate

As cardiac arrest occurred unexpectedly in over one third of the patients with myocardial infarction in our series after they had been in hospital for more than five days it seems advisable to concentrate the treatment of all patients with myocardial infarction in a well equipped special unit in a hospital

Summary

One hundred and thirty two successive episodes of cardiac arrest in 100 medical patients were treated by resuscitative procedures In 77 episodes ventricular fibrillation was evident, resuscitation was initially successful in 55 episodes Asystole was registered in 38 episodes of which ten were successfully treated An unknow rhythm was the cause of 17 episodes of cardiac arrest, of which eight responded to resuscitative measures The majority of the cases of ventricular fibrillation were treated by 1—5 direct current electrical countershocks Defibrillation led to asystole in 29 episodes of ventricular fibrillation but in most cases sinus rhythm was restored during continued resuscitation In 53 episodes the resuscitation was continued over 15 minutes and the treatment was successful in 30 % of the episodes The influence of the place where cardiac arrest occurs, intubation, various drugs and other factors on the success of resuscitation is discussed

Twenty seven patients survived and were discharged from hospital Age and sex were not found to influence the success of the resuscitation The series included 63 patients with acute myocardial infarction, 19 of these were discharged from hospital In the patients with myocardial infarction the first episode of cardiac arrest occurred during the first five days in hospital in 63 % and later in 37 %

At the time of the follow up examinations in September 1966, 23 of the patients were alive Up to this time, these patients had lived on average eight months after the cardiac arrest The results of the follow up examinations are presented

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From the First Department of Medicine (Head Pentti I Halonen M D) Helsinki University Central Hospital Helsinki and the Hospital Department (Head S Jokipii M D) Kustaankartano Home for the Aged, Helsinki Finland

Combined Parasympathetic and Beta-receptor Blockade as a Clinical Test

By

M H Frick, J Heikkilä and A Kahanpaa

Measurement of the heart rate is a simple but informative test included in every patient s clinical evaluation The significance of the measurement can be increased by using different procedures which change the patients physiological equilibrium, for instance muscular exercise postural changes, carotid sinus stimulation and respiratory maneuvres

Heart rate is also a variable often measured in pharmacological experiments and in therapeutic trials with various drugs A new approach was developed by Jose (16) who was the first using atropin and propranolol simultaneously to eliminate the heart from nervous and humoral influences The heart rate achieved after an intravenous injection of 0 2 mg kg of propranolol and 0 04 mg/kg of atropin was labelled by him as the intrinsic heart rate Preliminary data (15 16) showed that the contractile properties of the heart were positively correlated to this parameter A similar procedure has also been used by Sloman (19)

We report here our experience of the use of this test in a variety of patients with cardiac and other diseases

Material

A total of 233 patients were studied One hundred and fifteen were males and 118 females Their ages ranged from 16 to 91 years Ninety nine patients without anemia cardiac disease thyroid disease or febrile illnesses were used as controls to delineate the age distribution Seventy two patients had cardiac disease of varying etiology and formed a group The following additional groups were included 25 cases of thyroid disease 15 cases of anxiety state 16 cases with anemia and six cases with a febrile illness

Method

A fixed dose of 2 4 mg of atropin was used if the patients weight exceeded 60 kg If less a dose of 0 04 mg/kg was employed

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By

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Measurement of the heart rate is a simple but informative test included in every patient's clinical evaluation. The significance of the measurement can be increased by using different procedures which change the patient's physiological equilibrium, for instance muscular exercise, postural changes, carotid sinus stimulation and respiratory maneuvres.

Heart rate is also a variable often measured in pharmacological experiments and in therapeutic trials with various drugs. A new approach was developed by Jose (16) who was the first using atropin and propranolol simultaneously to eliminate the heart from nervous and humoral influences. The heart rate achieved after an intravenous injection of 0.2 mg/kg of propranolol and 0.04 mg/kg of atropin was labelled by him as the intrinsic heart rate. Preliminary data (15, 16) showed that the contractile properties of the heart were positively correlated to this parameter. A similar procedure has also been used by Sloman (19).

We report here our experience of the use of this test in a variety of patients with cardiac and other diseases.

Material

A total of 233 patients were studied. One hundred and fifteen were males and 118 females. Their ages ranged from 16 to 91 years. Ninety nine patients without anemia, cardiac disease, thyroid disease or febrile illnesses were used as "controls" to delineate the age distribution. Seventy two patients had cardiac disease of varying etiology and formed a group. The following additional groups were included: 25 cases of thyroid disease, 15 cases of anxiety state, 16 cases with anemia and six cases with a febrile illness.

Method

A fixed dose of 2.4 mg of atropin was used if the patient's weight exceeded 60 kg. If less, a dose of 0.04 mg/kg was employed.

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This dose of atropin was considered large enough to block parasympathetic activity As an expression of this tested by long run ECGs, the respiratory variation of the heart rate was completely abolished After preliminary trials a dose of 0 1 mg/kg of propranolol (Inderal® Imperial Chemical Industries, Ltd Alderly Park, Chesh) was selected Higher doses were well tolerated by the younger age groups, but in those over 70 years some unwanted side effects occurred mainly in the form of dizziness confusion and weakness The beta receptor activity is normally very low at supine rest (18) While evidently a major part of this activity was blocked the dose was not large enough to block all the activity as tested by isoproterenol challenge (12)

Atropin and propranolol were given intravenously in the same syringe over a period of two min The heart rate was measured either by auscultation or by ECG before and three min after the end of the injection

Duplicate tests at intervals of one to five days were made on 18 subjects The methodological error calculated from these duplicates was 2 8 beats/min or a coefficient of variation of 2 7 %

Results

The effect of sex and age distribution

Ninety nine patients were the basis of this evaluation Fifty four were males and 45 females their ages ranging from 18 to 85 years Medical history, clinical examination ECG, chest X ray, blood picture and protein bound iodine (PBI) determinations when required showed that these patients were free from cardiac disease anemia fever and thyroid disease None was receiving therapy known to deplete catecholamines from the tissues In spite of a normal ECG heart volume and configuration ten of the patients aged 66, 70 76 77, 79 80

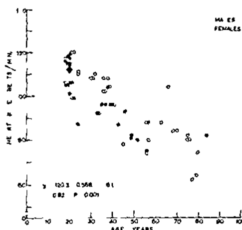


Fig 1 Correlation betwen the blocked heart rate and age

80, 81, 84 and 85 years were receiving digitalis as a part of the general supportive therapy to the aged

The age distribution of the blocked heart rate is exemplified by fig 1 It is evident that there was no significant difference between the male and female patients A linear correlation between the blocked heart rate and the age is evident from the regression equation of blocked heart rate = 120 3 — 0 588 × age ± 8 1 (S D) (r = — 0 82 P< 0 001)

This age distribution was the reference frame for subsequent evaluation of the effects of various diseases on the blocked heart rate

Thyrotoxicosis and anxiety state

The diagnostic criteria for thyrotoxicosis were a typical history and clinical picture with an elevated PBI In several cases also the 24 hour I^{131} uptake was informative The PBI ranged from 8 4 to 20 2 μg % The only case with established hypothyroidism had a PBI of

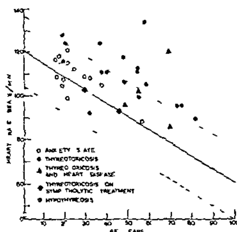


Fig 2 The blocked heart rate in patients with thyrotoxicosis and anxiety The normal regression and ± 2 S D are shown

1 0 μg %. Four of the thyrotoxic subjects had heart disease and were on digitalis One of the subjects was on guanethidine and another on reserpine treatment because of very rapid heart rates

An anxiety state was characterized by bouts of palpitations, rapid heart rates and sweating together with subjective symptoms of anxiety None had any specific signs of thyrotoxicosis and all had a normal PBI

Fig 2 demonstrates the blocked heart rates in these subjects Both cases on sympatholytic treatment were within the normal regression, as were three of the four cases with heart disease In the majority of the cases with plain hyperthyroidism the blocked heart rates exceeded the normal range The patient with hypothyroidism had a low normal response to the blockade In sharp contrast to the thyrotoxic subjects all patients with anxiety had heart rates within the normal regression Without blockade the mean heart rate of the thyrotoxic group

Table I Actual blocked and predicted normal blocked heart rates in thyrotoxicosis and anxiety state

	No of pats	Mean	S D	P
Thyrotoxicosis				
Actual	18	111 2	13 0	0 01
Predicted normal	18	91 3	11 7	0 01
Anxiety state				
Actual	15	108 8	8 0	—
Predicted normal	15	106 8	± 1	

was 108 and that of the patients with anxiety 106 beats min When the blocked heart rates of the thyrotoxic group without treatment were compared with the rates predicted from the equation relating the age and the blocked heart rate, a significant difference was observed (table I) No difference from the age adjusted blocked heart rates was observed in the group of anxiety state

Heart disease

The largest subgroup (33 cases) of the 72 patients with heart disease had arteriosclerotic and or hypertensive heart disease (AHHD) Nine were in atrial fibrillation and nine had unequivocal ECG signs of previous myocardial infarction Most of the cases had both ECG and X ray evidence of left ventricular strain Thirty one patients were on digitalis and four with hypertension were receiving sympatholytic treatment All but one were compensated at the time of the test

Fig 3 reveals that the blocked heart rates of this group were well within the normal range, two of the cases even ex-

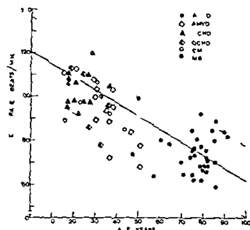


Fig 3 The blocked heart rate in patients with heart disease AHHD = arteriosclerotic and/or hypertensive heart disease AMVD = aortic and/or mitral valvular disease NCHD = nonobstructive congenital heart disease OCHD = obstructive congenital heart disease CMP — cardiomyopathy MG = miscellaneous group The normal variation with age is shown

ceeding the + 2 S D limit The only significantly depressed value was in the case in frank failure despite treatment with digitalis and diuretics

The subgroup of aortic and/or mitral valvular disease (AMVD) consisted of 14 patients Three were in atrial fibrillation and nine on digitalis treatment None was in clinical failure The blocked heart rates of this group tended to be low but only two cases exhibited significantly depressed values (fig 3)

The subgroup of non obstructive congenital heart disease (NCHD) consisted of seven cases of atrial septal defect of the secundum variety three cases with a patent ductus and one case with ventricular septal defect All were in sinus rhythm and only one was receiving digitalis All the blocked heart rates were within the normal range (fig 3)

Obstructive congenital heart disease (OCHD) was represented by three cases of valvular pulmonary stenosis and three cases of coarctation of the aorta All were in sinus rhythm and one on digitalis Two of the patients both with pulmonary stenosis had the blocked heart rates below normal (fig 3)

The subgroup of cardiomyopathy (CMP) consisted of five cases one of which had a muscular subvalvular pulmonary stenosis They were all in sinus rhythm and none was on digitalis One had a significantly depressed blocked heart rate (fig 3) The miscellaneous group (MG) contained one case with carditis, probably of virus origin one case with bouts of supraventricular tachycardia and one case with Wolf Parkinson White syndrome The WPW syndrome showed a low response to the blockade (fig 3)

Anemia and fever

Sixteen anemic subjects with hemoglobin (Hb) levels below 11 5 g/100 ml were included Only two cases exhibited

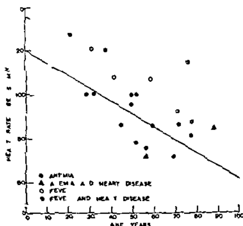


Fig 4 The blocked heart rate in patients with fever and anemia

blocked heart rates over the + 2 S D limit according to age (fig 4) One had a Hb value of 9 1 and the other one of 10 0 g/100 ml However several cases had Hb values as low as 7 0 g/100 ml with normal blocked heart rates The response of the febrile patients (fig 4) showed the same independence of the degree of fever

Discussion

A collection of relevant data on the effect of age on the heart rate (11) demonstrates a distinct decrease in both the basal and non basal heart rates from one to approximately 18 years Thereafter no significant change is evident Although this applies to values at rest, the maximal heart rates during exercise are clearly reduced in older age groups (20) When both atropin and propranolol were used to eliminate the neural and humoral control mechanisms a rather steep reduction in the heart rate with age was observed The regression equation y = 120 3 − 0 588 × age, is almost equal with the correlation obtained by Jose (16) y = 117 2 — 0 53 × age The difference of three beats min is probably related to a more complete beta blockade achieved by Jose using twice the dose of propranolol

The heart rate of a normal resting supine man is mainly governed by a parasympathetic restraint (18) in addition to the intrinsic rhythmicity of the pacemaker tissue Accordingly, the elevation of the heart rate observed in practically all age groups during the double blockade reflects abolition of the vagal restraint The significant inverse correlation of the blocked heart rate with age (fig 1) must be regarded as an expression of higher intrinsic fire off rate of the pacemaker tissue in the younger age groups This finding has many important implications to the understanding of tachycardias at different ages For instance a sinus tachycardia of 120 min in a subject aged 25 years signifies that the pacemaker is operating within its normal intrinsic range, whereas a similar rate in a subject aged 50 is definitely abnormal Conversely the therapeutic effect of atropin in states of low heart rate of sinus origin cannot be expected to be marked in the older age groups

Both thyrotoxicosis and anxiety are characterized by palpitations and rapid heart rates The differentiation between these two diagnoses may not be easy as persons with anxiety may have goitres and high PBI values may be found after iodine containing drugs contraceptive pills (8) and previous X ray investigations with iodinated contrast dyes An attempt has been made to differentiate anxiety and thyrotoxicosis using propranolol (22) but variance analysis showed no statistical difference in the heart rates Several attempts to estimate the role of sympathetic nervous system in the genesis of the cardiovascular manifestations of thyrotoxicosis (1 6 7 9 10 14 17 21 23) have led to controversy on whether thyroxine has a direct effect on the heart or whether the effect is mediated via the sympathetic nervous system The residual tachycardia after sympatholytes has been generally interpreted as incompleteness of the blockade The important finding of Herrlich et al (13) that thyroxin de

creases the acetylcholine content of the atria has been totally neglected

In the present study the attempt to distinguish thyrotoxicosis from anxiety included the use of atropin which thus should eliminate the possible effect of thyroxin via the atrial acetylcholine Results given in fig 2 show that the blocked heart rates of thyrotoxic patients were higher than those of patient with anxiety Although this difference was significant (table I) some overlapping occurred especially in co exi ting heart di ease or in patients on sympatholytic treatment The latter combination sug gests that the dose of propranolol was insufficient to block completely beta receptor activity Accordingly no conclu sions can be drawn about the mechanism of the thyrotoxic tachycardia The elevated blocked heart rates in thyro toxicosis after a propranolol dose of 0 2 mg kg (16) favor a direct action of thyroxin on the pacemaker tissue or an effect mediated through the elevated body temperature inherent to hyper thyroidism Nevertheless if patients with heart disease and those on sympatho ytic treatment are excluded the present test seems to differentiate well between anxiety and thyrotoxicosis We therefore sugge t that in considering a thyroid case a blocked heart rate exceeding 137 — 0 59 × age (beats min) in the absence of a febrile illness s gnificant anemia salicylate or glucocorticoid therapy (16) can be confidently con sidered as indicative of thyrotoxicosis The high reproducibility of the test is a further advantage in the follow up of the patient during therapy

The only case with hypothyroidism and a normal respon e to the blockade is corollary to the evidence that the hypokinesis of hypothyroidism is mainly due to a negative inotropy (1)

The blocked heart rates in the group with heart di ease showed the same age dependency as the controls (fig 3) In the light of evidence presented by Jose (16) this might indicate a gradual decrease in the contractility of the heart with age in any population studied So far as plain stroke volume can be considered an indicator of contractility, this assump tion is supported by the finding of Brandfonbrener et al (3) of a gradual decrease in stroke volume with age While Jose (16) found highly significant depressions from the normal regression in various groups of heart disea e this was not so evident in the present series (fig 3) Most of the cases with AHHD were in the older age groups and the blocked heart rates were within the normal variation In the younger age groups with predominantly AMVD NCHD and OCHD the blocked heart rates were in general below the control mean but still within the normal range Apparently as a group they had de pressed regression but in individual pa tients the test was not informative The fact that most of our patients were digitalized and given a different pro pranolol dose might explain the different response Nevertheless suggestive evi dence was found for the parallelism be tween the rate and the state of the myo cardium in the thyroid series three out of four cases with heart disease had low blocked heart rates and one case in the heart series had a significantly depressed value combined with heart failure

The series of anemic and febrile patients is rather small and the cases can be considered only as illustrative examples The normal blocked heart rates in the majority of the anemic patients are commensurate with the evidence that the hyperkinesis of anemia is mainly due to positive inotropy (2, 5) although also rapid heart rates are well established clinically The febrile cases exhibiting rapid blocked heart rates are probably examples of a direct effect of heat on the pacemaker tissue The effect of fever on the blocked heart rate apparently requires a separate study with special emphasis on the hitherto unexplained low heart rates in some viral and typhoid infections

Summary

The diagnostic value of the heart rate achieved after an intravenous injection of atropin and propranolol was studied in 233 patients Duplicate tests on same individuals revealed a coefficient of variation of 2 7 % The heart rate achieved the blocked heart rate, showed a significant negative correlation with the age via a regression of $y = 120.3 - 0.588 \times$ $\pm$ 8 1 (S D) Most of the subjects with thyrotoxicosis had elevated blocked heart rates whereas cases with anxiety fell within the normal regression

Patients with heart disease especially congenital and aortic and/or mitral valvular disease had depressed regression as a group For a single individual, however the test was not informative Some anemic subjects as well as some patients with fever had elevated blocked heart rates

The greatest practical use of this test will be in the investigation of patients with thyroid disease, especially when high protein bound iodine values are due to contamination Moreover the high reproducibility of the test is a further advantage in following the thyroid patient during therapy

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Chromosome Studies in Acute Leukaemia

III Chromosome constitution of bone marrow cells in 30 cases

By

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The demonstration of nuclear irregularities and abnormal mitotic figures in cancer cells at the end of the past century gave rise to speculations concerning a possible relationship between abnormal chromosome complements and initiation of cancer (2, 5) However not until recently have cytologic techniques been sufficiently improved so as to make a detailed study of the chromosome patterns of normal and neoplastic cells possible Following this it has been well established that the malignant cells of leukaemia and solid neoplasms frequently display abnormal chromosome complements

The present report deals with the results of cytogenetic studies performed during the past two years on bone marrow aspirates from 30 patients with acute leukaemia

Material and methods

The patients were unselected Fourteen were males and 16 were females Cytogenetic studies performed on bone marrow cells and/or cultured blood cells of ten of these patients (nos 1, 11 12 13 14 16 17, 25 26 28) have previously been published (8 9 10) Their ages ranged from 1/2 to 75 years Six of the patients were less than 14 years old Twenty five patients were untreated when first studied whereas the remainder had received therapy with prednisone 6-mercaptopurine or amethopterin Serial chromosome studies were performed in 14 patients

The series encompasses patients with lymphoblastic myeloblastic promyelocytic, monocytic and acute erythro-leukaemia (table I) The criteria used for differentiating between lymphoblastic and myeloblastic leukaemia were as follows "Blast

Table I Number of patients belonging to the various types of acute leukaemia

Type of leukaemia	No of pats
Lymphoblastic	6
Myeloblastic	17
Blast cell	1
Promyelocytic	1
Monocytic	2
Erythroleukaemia	3

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cells were classified as lymphoblasts when the chromatin was coarsely granular and the nuclei were round and relatively uniform with scanty cytoplasm and little variation in nucleocytoplasmatic ratio Cells with finely granular chromatin and wide variations with respect to cell size nuclear shape and nucleocytoplasmatic ratio were classified as myeloblasts The leukaemia was also considered myeloblastic if the primitive cells contained Auer rods and when a proportion of cells with blastic nuclei had cytoplasmatic granulation No distinction was made between myeloblastic and Naegeli type of monocytic leukaemia In one patient it was not possible to classify the blast cells as lymphoblasts or myeloblasts In monocytic leukaemia (Schilling type) young and ma ure monocytes were present in the marrow aspirate in highly increased numbers The marrow aspirate of the patient with promyelocytic leukaemia was characterized by an increased number of myeloblasts, atypical promyelocytes and myelocytes with promyelocytes as the dominating cell type Acute erythroleukaemia was diagnosed when the bone marrow was dominated by mye loblasts and clearly megaloblastic red cell precursors The classification of the material in the various types of acute leukaemia was made by same person throughout

The chromosomes of many leukaemic metaphases have a blurred appearance and are often poorly spread, and for this reason it may be impossible to give an exact ac count of the karyotypes of such metaphases Consequently an erroneous picture of the chromosome patterns in acute leukaemia may be achieved if only analysable meta phases are scored Therefore in the present series every metaphase has been scored in which the number of chromosomes could be counted Whenever possible a karyotypic analysis has been performed In each patient attempts were made to score 50 metaphases from each bone marrow preparation

As controls the marrow aspirates of ten patients who did not suffer from cancer

TABLE II Chromosomal findings in six cases of lymphoblastic leukaemia

Pat no	Age (yrs) Sex	Date	Total cells counted	Survival from diagnosis	Chromosome number				
					<40	40	41	42	43
1	26	6 1 65	25(7)[1]	15					
	♂	3 3 65	100(63)	months	1				
		1 12 65	50(14)						
2	2	30 10 65	50(13)	> 14					
	♂			months					
3	5/12	17 1 66	50(9)	10					
	♂			days					
4	54	22 7 66	22(3)	3					
	♀	8 8 66	50(10)	months					
5	9	27 7 66	18(5)	> 6					
	♂	10 9 66	50(20)	months					
6	53	17 9 66	8(2)	> 3 1/2					
	♂	13 10 66	22(7)	months					

[1] The figures in brackets indicate the metaphases analyzed
U = untreated
P = prednisone
6—MP = 6-mercaptopurine

or any haematologic disorder were cytogenetically investigated

For cytogenetic observations the marrow aspirates were treated according to the technique described by Tjio and Whang (19) with one modification For hypotonic treatment 20 ml sodium citrate (0 95 % in distilled water) was added to the bone marrow cells which had been divided among four test tubes

In eight of the patients in whom aneuploid marrow s emlines were present a bone marrow differential count was done Five hundred cells were counted in Giemsa stained smears of the same marrow aspirates which were used for cytogenetic study

Results

The results of the chromosome study in the leukaemic and normal individuals are *ummarized in tables II—V In* eleven patients a definitely abnormal cell line could be demonstrated The chromosomal findings in these cases are given in detail below In many of the cases in which no abnormal cell lines were demonstrated an increased number of aneuploid or pseudodiploid metaphases was found

When comparing tables II—IV with table V it is seen that in many of the leukaemic patients an increased number of metaphases with structural chromosome aberrations were present The abnormalities found were breaks of the chromatid and chromosome types acentric fragments and deletions Structural changes of the chromosomes were found in untreated patients as well as in patients receiving treatment with cytotoxic drugs

In nearly all the bone marrow aspirates studied in relapse the chromo-

44	45	46	47	48	49	>49	Polyploid cells (%)	No of metaphases with structural *abnormalities*	Treatment
		25					0	1	P
1	5	91	1	1			0	7	6-MP
	1	18	31				0	1	P+6—MP
	4	43	3				0	8	U
	5	44	1				0	5	U
1	1	20					0	0	U
2	5	42	1				0	5	P+6—MP
		18					0	0	U
	2	48					0	3	6—MP
	2	6					0	2	U
1	1	20					0	2	P

TABLE III Chromosomal findings in 18 cases of myeloblastic leukaemia

Pat no	Age (yrs) Sex	Date	Total cells counted	Survival from diagnosis	Chromosome number				
					<40	40	41	42	43
7	45	22 9 64	39(19)	2 1/2	3		1	1	3
	♀	27 10 64	40(33)	mos	2	1	1		1
		28 10 64	35(17)						
8	29	3 6 65	44(9)	>41		1			1
	♀	16 11 66	50(14)	mos	1				
9	38	5 11 64	25(12)	2 1/2					1
	♀			mos					
10	10	5 2 65	38(7)	7					
	♂			mos					
		3 6 65	50(9)						
11	49	23 7 65	50(26)						
	♀	29 9 65	50(19)	11					1
		5 1 66	50(16)	mos					
		24 3 66	50(16)			1			1
12	56	25 6 65	50(15)	3			2		1
	♂			days					
13	75	2 10 65	50(21)	1 1/2					
	♂	9 11 65	50(10)	mo					3
14	68	12 11 65	50(20)	2 1/2					1
	♀			mos					
		18 11 65	50(21)					3	
15	30	10 12 65	50(14)	9				1	1
	♀	11 3 66	50(7)	mos					
16	70	23 12 65	44(0)	1 1/2	2	4	21	9	5
	♀			mo					
17	35	21 2 66	50(0)	1 1/2	1		2		3
	♂	1 4 66	50(0)	mo		1	2	3	17
18	38	7 5 66	50(13)	11	1				
	♀			mos					
19	71	4 6 66	50(12)	14					1
	♀			days					
20	8	7 6 66	29(2)	2					
	♂			weeks					
21	42	8 7 66	50(17)	>9 1/2					
	♂			mos					
22	48	9 8 66	50(24)	2 1/2				1	1
	♀			mos					
23	54	5 11 66	11(2)	>2					
	♀			mos					
24	73	2 12 66	27(3)	>1					
	♂			mo					

[1] The figures in brackets indicate the number of metaphases analyzed

44	45	46	47	48	49	>49	Polyploid cells (%)	No of metaphases with structural abnormalities	Treatment
3	4	24					0	2	U
2	1	32					3	5	6—MP
1	3	30	1				0	5	U
2	4	36					0	1	U
1	2	46					0	0	P
	4	20					0	1	U
2	4	32					0	0	U
4	38	8					0	13	U
	4	46					0	0	U
	2	47					0	6	6—MP
	3	47					0	7	6—MP
	3	45					0	1	A
4	3	40					0	2	U
4	6	31	9				0	4	U
1	17	24	5				0	5	P+6—MP
2	4	43					0	5	U
4	9	32	2				0	2	U
3	4	40				1	0	6	U
	9	37	3			1	0	3	A
1		2					0	17	U
42	2						3	9	U
24	1	2					10	8	6—MP
	2	42	4			1	0	4	6—MP+A
1	3	45					0	0	U
	2	10	16	1			0	4	U
	1	49					0	2	P+6—MP
2	7	38	1				0	2	U
	2	9					0	1	U
1	2	23	1				0	0	U

U = untreated P = prednisone 6—MP = 6-mercaptopurine A = amethopterin

TABLE III Chromosomal findings in 18 cases of myeloblastic leukaemia

Pat no	Age (yrs) Sex	Date	Total cells counted	Survival from diagnosis	Chromosome number <40	40	41	42	43
7	45	22 9 64	39(19)	2 1/2	3		1	1	3
	♀	27 10 64	40(33)	mos	2	1	1		1
		28 10 64	35(17)						
8	29	3 6 65	44(9)	>41		1			1
	♀	16 11 66	50(14)	mos	1				
9	38	5 11 64	25(12)	2 1/2					1
	♀			mos					
10	10	5 2 65	38(7)	7					
	♂			mos					
		3 6 65	50(9)						
11	49	23 7 65	50(26)						
	♀	29 9 65	50(19)	11					1
		5 1 66	50(16)	mos					
		24 3 66	50(16)			1			1
12	56	25 6 65	50(15)	3			2		1
	♂			days					
13	75	2 10 65	50(21)	1 1/2					
	♂	9 11 65	50(10)	mo					3
14	68	12 11 65	50(20)	2 1/2					1
	♀			mos					
		18 11 65	50(21)					3	
15	30	10 12 65	50(14)	9				1	1
	[illegible]	11 3 66	50(7)	mos					
16	70	23 12 65	44(0)	1 1/2	2	4	21	9	5
	[illegible]			mo					
17	35	21 2 66	50(0)	1 1/2	1		2		3
	[illegible]	1 4 66	50(0)	mo		1	2	3	17
18	38	7 5 66	50(13)	11	1				
				mos					
19	71	4 6 66	50(12)	14					1
	[illegible]			days					
20	8	7 6 66	29(2)	2					
	♂			weeks					
21	42	8 7 66	50(17)	>9 1/2					
	♂			mos					
22	48	9 8 66	50(24)	2 1/2				1	1
	♀			mos					
23	54	5 11 66	11(2)	>2					
	♀			mos					
24	73	2 12 66	27(3)	>1					
	♂			mo					

[1] The figures in brackets indicate the number of metaphases analyzed

leukaemia (nos 28—29) and blast cell leukaemia (no 30)

							Polyploid cells	No of metaphases with structural	Treatment
44	45	46	47	48	49	>49	(%)	abnormalities	
1	1	1					7	50	U
							1	45	U
1							0	48	U
	3	45				1	0	0	U
	3	47					0	2	U
1	2	44	2				0	9	6—MP
	4	43				1	0	0	P
2	41	2					0	2	P
5	40	1					0	4	P+6—MP
	1	43	1		2		4	5	U
1	1	11					0	0	U
	3	40				7	0	3	U

U=untreated P=prednisone 6-MP=6-mercaptopurine

somes had a blurred appearance (fig 1) In a few patients bridges of chromatin were seen between different chromosomes (fig 2) In most bone marrow preparations metaphases with a blurred appearance of the chromosomes as well as metaphases with a normal appearance were met In patients with aneuploid cell lines in the bone marrow it was regularly found that chromosomes in metaphases belonging to aneuploid stem lines were blurred, whereas chromosomes in most diploid metaphases were normal in appearance The chromosomes in mar

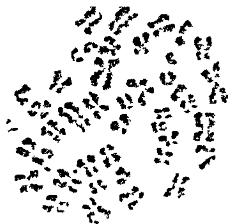


Fig 1 Hypodiploid marrow metaphase from patient 25 with ill-defined and blurred appearance of chromosomes

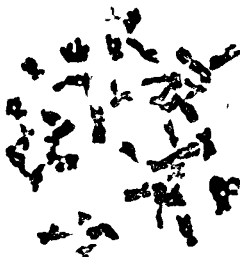


Fig 2 Marrow metaphase from patient 8 with bridges of chromatin between the blurred chromosomes

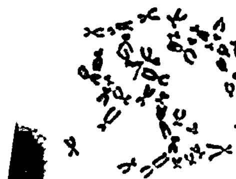


Fig 3 Diploid marrow metaphase from patient 11 in remission with well-defined chromatids

TABLE VI Bone marrow differentials and chromosomal findings in eight patients with acute leukaemia and abnormal stemlines

Pat no	Date	Bone marrow differential (per cent) Blast cells	Promyelocytes	Myelocytes	Metamyelocytes	Granulocytes	Lymphocytes	Erythroblasts	Other cell types	Aneuploid metaphases (%)
1	1 12 65	97	—	—	—	—	1	2	—	62
13	2 10 65	28	10	15	6	20	11	5	5	20
16	23 12 65	41	3	3	1	4	5	43	—	95
17	21 2 66	16	21	3	2	13	11	34	—	98
	1 4 66	28	2	2	1	3	9	55	—	96
20	7 6 66	94	—	—	—	4	2	—	—	59
24	2 12 66	73	—	6	2	6	—	12	1	96
25	10 5 65	7	2	4	5	7	7	46	22	98
	9 6 65	14	2	4	3	4	3	55	15	100
	31 7 65	90	2	4	1	1	—	—	2	100
28	30 6 65	39	9	13	7	20	10	2	—	96
	10 11 65	25	1	11	4	19	16	24	—	98

row metaphases obtained from patients in remission had a normal chromatin structure (fig 3)

The results of the marrow differentials in the patients with abnormal stemlines in the marrow aspirates are compared to the percentage of abnormal metaphases in table VI Comparison of this table with tables II—IV shows that in only two instances (patient no 13 and in the

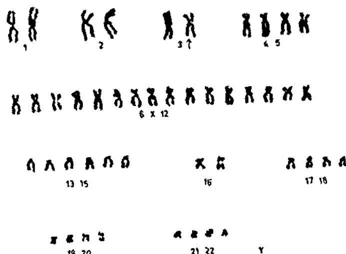


Fig 4 Karyotype of diploid marrow metaphase from patient 7 showing a partial deletion of two arms of a chromosome no 3

third marrow aspirate of patient no 25) there was a rough correlation between the percentage of abnormal white cell precursors in the direct marrow smears and the percentage of abnormal metaphases in the chromosome preparations In the other patients, the abnormal metaphases were proportionally either fewer (cases nos 1 and 20) or more numerous (cases nos 16, 17 24, 25 28) than the abnormal white cell precursors

Particulars of chromosomal findings in eleven patients with abnormal stemlines in the bone marrow aspirates

Case 1

Twenty six year old man with lymphoblastic leukaemia (table II) diagnosed November 1964 died February 1966 Therapy prednisone 5 12 64—22 1 65 20 10 65—28 2 66 6-mercaptopurine 6 1 65—29 12 65 amethopterin 29 12 65—6 2 66 (daily) 7 2 66—22 2 66 (weekly)

The first marrow aspirate was obtained in January 1965 when the patient was in partial remission Only diploid metaphases were found A marrow aspirate obtained two months later when the patient was still in partial remission had a sharp diploid mode One hundred metaphases were counted only one of which had the abnormal karyotype present in relapse In December 1965 when the bone marrow was completely dominated by lymphoblasts a mode of 47 chromosomes was found The hyperdiploidy was due to a supernumerary chromosome in group 13—15

Case 7

Forty five year old woman with myeloblastic leukaemia (table III) diagnosed September 1964 died December 1964 Therapy 6-mercaptopurine 22 9 64—1 12 64 prednisone 13 11 64—1 12 64 amethopterin 2 12 64—7 12 64

In the marrow aspirate obtained before therapy in September 1964 a pseudodiploid cell line was found Nineteen cells were karyotyped in 16 a partial deletion of the arms of one of the chromosomes no 3 was present (fig 4) A translocation of the deleted material to another chromosome could not be ascertained Three metaphases were normal In the second marrow preparation obtained five weeks later the same

pseudodiploid mode was found Thirty three cells were karyotyped 30 of which contained the deleted chromosome no 3

Case 11

Forty nine year old woman with myeloblastic leukaemia (table III) diagnosed May 1965 died April 1966 Therapy 6 mercaptopurine 4 6 65—22 6 65 15 9 65—3 3 66 amethopterin 3 3 66—11 4 66 (once or twice weekly) prednisone 30 3 66—16 4 66

In June 1965 the marrow aspirate obtained before therapy had a mode of 45 chromosomes The hypodiploidy was due to a chromosome missing in group 6 X 12 and 21—22 respectively A supernumerary chromosome was present in group 13—15 The second marrow aspirate when the patient was in partial remission, had a sharp diploid mode Only two metaphases with the above mentioned abnormal karyotype were found The third marrow aspirate in which about 40 % of the nucleated cells were myeloblasts also had a sharp mode of 46 chromosomes Thirty seven of the diploid metaphases counted had a normal female karyotype In two metaphases an extra chromosome was present in group 13—15 whereas one chromosome was missing in group 21—22 No cell with the original abnormal hypodiploid karyotype was found The fourth and fifth aspirates which were dominated by myeloblasts had pseudodiploid modes as most of the metaphases with 46 chromosomes contained a supernumerary chromosome in group 13—15 whereas one chromosome was missing in group 21—22

Case 13

Seventy four year old man with myeloblastic leukaemia (table III) diagnosed October 1965 died November 1965 Therapy prednisone 2 10 65—11 11 65 6 mercaptopurine 9 10 65—11 11 65

In the marrow aspirate obtained before therapy in October 1965 about 30 % of the cells were myeloblasts In the chromosome preparations of the same aspirate a diploid mode was found However about 20 % of the metaphases contained 47 chromosomes due to an extra chromosome belonging to group 6 X 12 In a marrow aspirate obtained a few days before death half of the cells were myeloblasts A diploid mode was still present with only 10 % of the metaphases being hyperdiploid However about 35 % of the metaphases now contained 45 chromosomes Due to a very blurred appearance of the chromosomes of these metaphases only two could be analyzed in both of which a chromosome of group 16—18 was missing

Case 16

Seventy year old woman with myeloblastic leukaemia (table III), diagnosed December 1965 died February 1966 Therapy 6 mercaptopurine 24 12 65—26 1 66

The marrow aspirate obtained at admission in December 1965 was dominated by myeloblasts and red cell precursors In the chromosome preparations of the same aspirate all metaphases except two were hypodiploid and 21 of them contained 41 chromosomes None of the abnormal metaphases could be analyzed Thirty two cells contained a marker chromosome In most cases a large acrocentric chromosome with the size of a chromosome no 1 was found In a few cells however a ring chromosome with the size of one of the chromosomes of group 6 X 12 was present

Case 17

Thirty five year old man with promyelocytic leukaemia (table III) diagnosed February 1966 died April 1966 Therapy 6 mercaptopurine 5 3 66—4 4 66 prednisone 8 3 66—6 4 66

The dominating cell types in the bone marrow aspirate obtained at admission were erythroblasts and promyelocytes Many myeloblasts and reticulum cells were seen In the chromosome preparations about 80 % of the metaphases contained 44 chromosomes none of which could be karyotyped In about 80 % of the bone marrow metaphases a marker chromosome was present viz a small acrocentric or metacentric chromosome half the size of a chromosome of group 21—22 A bone marrow aspirate obtained five weeks later had two modes 44 and 43



Fig 5 Marrow metaphase from patient 20 containing 47 chromosomes with a large acrocentric chromosome

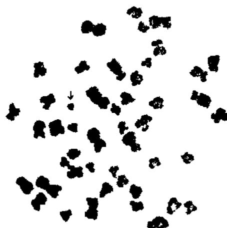


Fig 6 a Marrow metaphase from patient 21 containing 46 chromosomes and a small acentric fragment A supernumerary chromosome is present in group 16—18 and one chromosome is missing in group 6 X 12

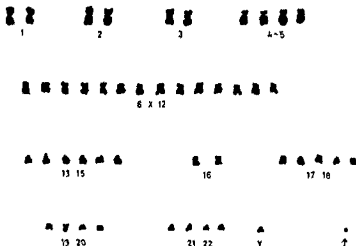


Fig 6 b Karyotype of the marrow metaphase of fig 6 a

chromosomes No metaphases could be analyzed The small marker chromosome could be seen in 20 % of the metaphases

Case 20

Eight year old boy with myeloblastic leukaemia (table III) diagnosed June 1966 died June 1966 Therapy prednisone 7 6 66—18 6 66 6 mercaptopurine 13 6 66—17 6 66

The marrow aspirate which was completely dominated by myeloblasts had a stemline of 47 chromosomes The chromosomes of all metaphases had a very blurred appearance so that none of the hyperdiploid cells could be analyzed Two of the diploid cells were analyzed These had a normal male karyotype In fourteen of the metaphases a marker chromosome was present viz a large acrocentric chromosome with the size of a chromosome no 6 (fig 5) It may be mentioned that in

February 1966 the patient had been treated with ionizing radiation (2500 r) towards the groin for a lymphosarcoma The leukaemia however was clearly myeloblastic

Case 24
Seventy three year old man with myeloblastic leukaemia (table III) diagnosed December 1966 died February 1967 Therapy prednisone 4 12 66—7 2 67, 6 mercaptopurine 4 12 66—7 2 67

A chromosome study of the bone marrow performed at the time of diagnosis revealed that the bone marrow cells had a pseudodiploid mode Twenty seven metaphases were scored, in 26 of which a small acentric fragment half the size of one of the chromosomes of group 21—22 was seen (fig 6) Probably the fragment originated from a chromosome of group 6-X-12 as in three cells which could be analyzed a chromosome of this group was missing whereas an extra chromosome with the size of the chromosomes of group 16—18 was seen The bone marrow was dominated by myeloblasts However 12 % of the nucleated cells were erythroblasts

Case 25
Seventy five year old woman with erythroleukaemia (table IV) diagnosed May 1965 died August 1965 Therapy 6 mercaptopurine 31 7 65—2 8 65

The first two marrow aspirates were dominated by megaloblasts and macronormoblasts Eighty four—ninety six % of the mitotic figures belonged to the erythrocytic series In both aspirates a mode of 42 chromosomes was found Most metaphases contained a ring chromosome The third marrow aspirate was completely dominated by myeloblasts 100 % of the mitoses occurring in the white cell series However the same abnormal mode was still found The cytogenetic findings in this patient have been reported in detail elsewhere (8)

Case 28
Fifty year old man with monocytic leukaemia (table IV) diagnosed March 1964 died March 1966 Therapy prednisone 9 5 64—10 2 66, 6 mercaptopurine 29 9 65—5 1 66 and 29 1 66—1 3 66

A chromosome study was not performed until June 1965 15 months after the diagnosis was made The bone marrow had a mode of 45 chromosomes the hypodiploidy being due to a chromosome missing in group 6-X-12 The bone marrow was dominated by young and mature monocytes A marrow aspirate obtained in November 1965 had the same abnormal mode and monocytes were still the dominant cell type However 42 % of the mitotic figures in the direct marrow smear belonged to the erythrocytic series

Case 30
Four year old girl with blast cell leukaemia (table IV) diagnosed October 1965 died November 1965 Therapy prednisone 11 10 65—7 11 65 6 mercaptopurine 11 10 65—7 11 65

The chromosomes of the bone marrow cells were studied at the time of diagnosis A mode of 46 chromosomes was found However, seven of 50 metaphases scored had a considerably increased number of chromosomes with a blurred appearance but could not be counted exactly However, in five metaphases the chromosome number was between 60 and 65 chromosomes Probably these cells constitute an abnormal stemline The direct smear from the same marrow aspirate was completely dominated by blast cells

Discussion

Ford et al (4) in 1958 were the first to demonstrate abnormal chromosome patterns in acute leukaemia Since then many reports have dealt with cytogenetic studies in patients with acute leukaemia The presence of abnormal stemlines and structural chromosome aberrations has been the most characteristic finding (3, 7 14 18) Moreover in many patients

without abnormal stemlines in the marrow an increased number of aneuploid and pseudodiploid metaphases has been demonstrated (1, 7) However, completely negative findings have been described in several patients (7, 18)

1 Abnormal stemlines

As an increasing number of patients are cytogenetically investigated a few common patterns seems to emerge, although no chromosome abnormality specific for acute leukaemia has been demonstrated Thus Sandberg et al (18) in their series of 75 patients with acute leukaemia found that aneuploidy in lymphoblastic leukaemia was invariably hyperdiploid, whereas abnormal stemlines in myeloblastic leukaemia were hypodiploid except in a small group of elderly patients who had a double abnormal cell population in the bone marrow, one of which was hyperdiploid As regards lymphoblastic leukaemia these results are consistent with most other reports Thus in a review of the literature we have been able to find only two patients with lymphoblastic leukaemia and a hypodiploid stemline (7) In myeloblastic leukaemia, however, the occurrence of hypodiploid and hyperdiploid stemlines is about equal

In remission the abnormal metaphases either disappear from the bone marrow or are greatly reduced in number, and normal diploid modes are present (14, 15, 18) Thus Reisman et al (15) studied consecutive bone marrow specimens of eight children with acute leukaemia during repeated relapses and intervening remissions Abnormal stemlines were consistently found during the active stages of the disease whereas in remission a normal diploid mode was present In subsequent relapse the original abnormal stemline reappeared in the marrow aspirates of the patients In two cases evolution of a secondary abnormal stem line was also seen

In the present series two patients (nos 1 and 11) with aneuploid stemlines in the marrow during relapse obtained a remission Although the latter was only partial in both cases, normal diploid modes with only a few abnormal metaphases were found Thus in case 1 only one abnormal metaphase similar to those present in relapse was found among 100 metaphases scored In both two patients the percentages of blast cells in the direct marrow smears which were obtained from the same bone marrow aspirates as the diploid chromosome preparations varied from 13 to 27 % and 11 to 40 % respectively The failing correlation between the number of blast cells and aneuploid metaphases in the marrow aspirates of these two patients is probably due to the considerably lower mitotic index of leukaemic cells than of normal bone marrow cells (17) At the same time the leukaemic mitotic figures are likely to be somewhat underestimated in the counts from the chromosome preparations because of the blurred appearance of their chromosomes The present findings indicate that failure to demonstrate any aneuploid metaphases in the bone marrow aspirates of leukaemic patients during remission cannot be taken as a proof of a complete suppression of the 'leukaemic' cells in the bone marrow as has also been emphasized by Reisman et al (15)

In most patients of the present series the failure to demonstrate a correlation between the percentages of abnormal white precursors in the direct marrow smears and of aneuploid metaphases in the chromosome preparations cannot be explained by the lower mitotic index of "leukaemic" cells, as in some patients rather few morphologically abnormal white cell precursors were found in the marrow aspirate, while most metaphases were abnormal. This finding suggests that cell types other than the "leukaemic" blast cells may contribute to the abnormal stemlines. Evidence has recently been presented that abnormal karyotypes are present not only in the "leukaemic" blast cells but also in the red cell precursors of some patients with acute leukaemia (9).

In a few patients in the present series two abnormal stemlines were found. Thus in patient no. 11 a stemline with 45 chromosomes was present in the bone marrow at admission. In remission a normal diploid mode was found. In relapse a pseudodiploid stemline—closely related to the former abnormal stemline—emerged. This evolution of a new clone of cells is probably due to the fact that the pseudodiploid stemline was more resistant to the cytotoxic drug employed than the hypodiploid stemline. The pseudodiploid clone may have been present in the first marrow aspirate although it could not be detected, or it may have arisen from the hypodiploid stemline due to a non disjunction or chromosome duplication.

Chromosome studies are usually not of diagnostic aid in acute leukaemia because some cases have normal chromosomal patterns. However, in a few cases of the present series the chromosomal findings proved to be of diagnostic value. Thus in patients nos. 17 and 25 it could not initially be decided from clinical and bone marrow studies whether the patients suffered from acute leukaemia or from a chronic infection or pernicious anaemia, (atrophic glossitis and low serum B_{12} concentration were also present), respectively. The presence of abnormal stem lines in the bone marrow excluded the latter two possibilities. The course of the disease confirmed the diagnosis of acute leukaemia.

2. Unspecific aneuploidy

In the present paper "unspecific aneuploidy" indicates the increased number of aneuploid and pseudodiploid metaphases in the marrow of many leukaemic patients in which abnormal stemlines cannot be demonstrated. The abnormal metaphases do not belong to a particular stemline as metaphases with the same mode have different karyotypes. The missing or supernumerary chromosomes belong to different chromosome groups.

Unspecific aneuploidy is probably secondary to the leukaemic process. In most cases the phenomenon is likely to be due to an increased fragility of the "leukaemic" metaphases resulting in a random loss of chromosomes from different groups. However, this phenomenon cannot explain the increased number of hyperdiploid or pseudodiploid metaphases. Probably the hyperdiploid and part of the hypodiploid cells result from a tendency of the leukaemic cells to non disjunction and anaphase lag.

3 Structural abnormalities of the chromosomes

In the present series structural chromosome aberrations were present in untreated patients as well as those receiving cytostatic therapy In the latter, some of the aberrations are probably produced by the antileukaemic treatment (11, 13)

The mechanisms which are responsible for the production of the structural chromosome aberrations in marrow cells of leukaemic patients are unknown They could be manifestations of a disordered DNA synthesis in the leukaemic cells, as similar aberrations are produced by a number of chemical agents which interfere with DNA synthesis, viz de-oxyadenosine (6), arabinosylcytosine (6), arabinosyladenine (12), 6 mercaptopurine (13), azathioprine (11), and the folic acid antagonists (11, 16)

Do cytogenetic studies in acute leukaemia give any information about the nature and pathogenesis of leukaemia?

1 The facts which have been presented concerning the distribution of the abnormal stemlines and metaphases with either blurred or structurally normal chromosomes in relapse and remission suggest that in patients with and without abnormal stemlines in the bone marrow the leukaemic cell populations are constituted by the aneuploid metaphases and the diploid metaphases with blurred chromosomes, respectively Diploid metaphases with structurally normal chromosomes probably belong to a non leukaemic population of cells which exists pari passu with the leukaemic cells In remission leukaemic cells are replaced by morphologically and cytogenetically normal cells

2 The demonstration of chromosome abnormalities in the erythroid precursors in the bone marrow aspirates of patients with acute leukaemia indicate that both the erythroblasts and blast cells are affected by the leukaemic process and thus probably are derived from a common pool of stemcells

3 Cytogenetic studies in acute leukaemia have not shown whether the abnormal stemlines are primary and thus of aetiologic significance or whether they are secondary but contribute to the evolution of the leukaemic process The absence of visible chromosome aberrations in some patients with acute leukaemia has been taken as evidence that abnormal chromosome complements do not play a role in the aetiology of this condition This objection is not valid however, as changes indetectable to light microscopy may be present in these cases Minor chromosome aberrations which are easily demonstrated in the smaller chromosome groups—such as the Ph[1] chromosome in chronic myelocytic leukaemia—may not be detected by the present rather crude techniques if present on the large chromosomes

It is now well established that abnormal chromosome complements frequently occur in patients with acute leukaemia and may antedate pathological changes in the bone marrow diagnostic of acute leukaemia (18) The presence of characteristic and usually stable abnormal stemlines in the bone marrow and the reappearance of identical or closely related karyotypes in relapse indicate that

the chromosome abnormalities are of significance for the maintenance and progression of the leukaemic process However, at present no definite statement of their role in the causation of acute leukaemia can be made

Summary

Cytogenetic studies of bone marrow cells from 30 patients with acute leukaemia are reported Abnormal stemlines were present in eleven cases An increased number of aneuploid cells not belonging to any abnormal stemline and structural changes of the chromosomes were found in most patients Two patients with abnormal stemlines in the bone marrow obtained a remission during which normal diploid modes were present

In the patients with abnormal stem lines no correlation was found between the percentage of aneuploid metaphases in the chromosome preparations and the percentage of blast cells in the direct marrow smears from the same aspirates

It is concluded that cytogenetic studies in acute leukaemia may give valuable information concerning the nature and pathogenesis of acute leukaemia although at present no definite statement of the role of chromosome abnormalities in the causation of leukaemia can be made

Acknowledgement

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Low Excretion of Fecal Bile Acids in a Family with Hypercholesterolemia

By

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Hypercholesterolemia, particularly the familial type is frequently associated with xanthomatosis and atheromatous heart disease Its origin has therefore been extensively studied, but no conclusive explanation has emerged (10) Theoretically an excessive influx of cholesterol to the body (endogenous synthesis and intestinal absorption) or a decreased catabolism of cholesterol (low excretion as neutral sterols and bile acids into feces) are two major factors that could lead to hypercholesterolemia Improved analytical methods for fecal steroids and dietary sterols (8 15) have made possible to study them in more detail A group of normocholesterolemic controls and a family with hypercholesterolemia and xanthomatosis have now been studied with the sterol balance technique The results show that hypercholesterolemic subjects excreted on a solid food diet subnormal amounts of bile acids into feces, whereas the neutral steroid output was within normal limits

Submitted for publication May 11 1967

Material and methods

The material consisted of eight hypercholesterolemic patients of the same family including one brother and seven sisters (two post menopausal) aged 31—50 years All of them had either xanthelasmata or tendon xanthomata but no heart failure or clinical and electrocardiographic signs of coronary heart disease At the time of admission to hospital serum cholesterol ranged from 305 mg/100 ml to 640 mg/100 ml falling during hospitalization by some 10 % Control material comprised 19 normocholesterolemic patients of about the same age range (five out of the nine women were post menopausal) hospitalized for different reasons such as hypotension mild hypertension healed pyelonephritis arthralgia without signs of active inflammation neurocirculatory dystony compensated rheumatic heart disease and biliary dyskinesia

All the patients were hospitalized for at least three weeks and were put on a solid food diet with caloric cholesterol and plant sterol intake as constant as possible To measure fecal flow rate (18 19) 600 mg of chromic oxide (Cr_2O_3), divided into three doses were given daily to each patient Dietary β sitosterol was used as another marker of fecal flow rate because it is poorly absorbable (7) and its possible degradation during intestinal passage is proportional to that of cholesterol (1) Consequently to measure average β sitosterol and also cholesterol intake, nine 24 hr food collections were carried out during the experimental period Analysis of these diets showed that daily β sitosterol intake was 103 $\pm$ 3 (SE) mg The average value was used for the calculation of fecal flow rate

Two day stool collections extending over six consecutive days were made in 12 patients during the third week Since in three cases the average 24 hr steroid excretion determined from these two day collections deviated non systematically from that obtained either on the basis of Cr_2O_3 or β sitosterol fecal steroids were calculated in relation to those markers and not to calendar time Therefore, from three to four fecal samples instead of total collections were obtained from the rest of patients for steroid and Cr_2O_3 analysis

Dietary cholesterol and β sitosterol (other plant sterols are not included) were determined from an aliquot of the homogenised 24 hr food collection (15) Another saponified aliquot was acidified and extracted with heptane Total dietary fat was determined from this heptane extract gravimetrically it ranged from 74 to 97 g/24 hrs Fatty acid composition was not determined Fecal cholesterol, β sitosterol (including their respective coprostanol and coprostanone derivatives) and bile acids were determined as presented earlier (8 15) Fecal Cr_2O_3 was measured after sulfuric acid perchloric acid digestion (4, 7 18) Daily excretion of bile acids and cholesterol was calculated as follows mg of steroid per g of fecal homogenate per mg of Cr_2O_3 (or β sitosterol) per g of fecal homogenate × daily intake of Cr_2O_3 (or β sitosterol) Serum cholesterol was measured according to Pearson at al (17) and triglycerides with the method of Carlson (5)

Results

Serum cholesterol and triglycerides as well as the results of sterol balance studies on control and hypercholesterolemic patients are presented in table I Mean daily cholesterol intake was 312 mg with a range from 275 to 373 mg

Fecal bile acid excretion was significantly lower in hypercholesterolemics (154 mg/24 hrs) than in controls (220 mg/24 hrs) Corresponding cholesterol (450 mg and 505 mg) and total steroid (605 mg and 721 mg) values did not differ significantly from each other The net sterol balance, average daily intake of cholesterol minus the sum of average daily output of cholesterol plus bile acids, was negative, 409 mg for controls and 293 mg for hypercholesterolemics Since these figures represent the net daily losses of cholesterol from the body on steady state they equal to the daily formation of cholesterol within the body It is thus apparent that under the conditions of the present study the endogenous synthesis of cholesterol was rather low particularly in patients with hypercholesterolemia That the latter subjects appear to excrete more of their fecal steroids as neutral steroids is shown by the ratio cholesterol/bile acids that in hypercholesterolemia was higher (3 28) than normal (2 35)

Ahrens (1) has pointed out that degradation of cholesterol occurring occasionally during intestinal passage is

TABLE I Sterol balance in control subjects and in members of a family with hypercholesterolemia

Group	Serum triglycerides	Serum cholesterol	Cholesterol intake	Fecal excretion[1]			Sterol balance	Fecal cholesterol/ bile acids
				Cholesterol	Bile acids	Total		
	(mg/100 ml)	(mg/100 ml)	(mg/24 hrs)	(mg/24 hrs)	(mg/24 hrs)	(mg/24 hrs)	(mg 24 hrs)	
Controls (19)	[2]127±20	234±10	312	505±45	220±16	721±55	−409	2.35±0.16
Hypercholesterolemics (8)	100±11	450±37	312	450±48	154±14	605±51	−293	3.28±0.33

[1] Values are obtained by using chromic oxide (600 mg/day) to mark fecal flow rate
[2] Determined from nine subjects Mean ± SE
Values in parentheses indicate the number of subjects

proportional to that of plant sterols and hence could be corrected by the recovery of dietary plant sterols (but not of Cr_2O_3) in feces Therefore fecal steroid excretion was related not only to recovered Cr_2O_3 but also to β sitosterol recovered in feces The values obtained with β sitosterol as an internal standard of fecal flow rate tended to be higher than those obtained on the basis of Cr_2O_3 Yet fecal bile acid excretion was significantly lower in hypercholesterolemics (166±20 mg/24 hrs) than in controls (247±23 mg/24 hrs) The outputs of cholesterol (486±33 mg and 568±61 mg/24 hrs) and total steroid (652±44 mg and 814±76 mg/ 24 hrs) were again of the same magnitude in both groups The net negative sterol balance was now 340 mg and 502 mg respectively

Fecal bile acids and cholesterol showed (fig 1) a positive correlation (r=0 54) in controls but did not correlate at all in hypercholesterolemics (r=0 09) The spots of control males and females are evidently scattered equally demonstrating that the fecal steroid excretion at least in the present material is not affected by sex, nor do the values for pre- and post menopausal women differ

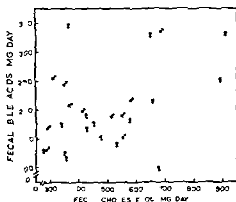


Fig 1 Correlation of fecal bile acids to fecal cholesterol (including coprostanol and coprostanone) in controls (open circles r = 0 54) and in members of a family with hypercholesterolemia (filled circles r = 0 09) Each point is an average of three to four determinations

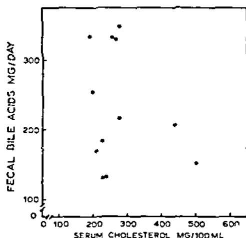


Fig 2 Correlation of fecal bile acids to serum cholesterol in the same patients as in fig 1 r = 0 20 in controls (open circles) r = —0 71 in hypercholesterolemics (filled circles)

Since the low excretion of fecal bile acids might conceivably have resulted in hypercholesterolemia fecal bile acids were plotted against serum cholesterol (fig 2) No correlation was found between these parameters in controls (r= 0 20) In hypercholesterolemic patients, however a negative correlation appeared to be present (r= —0 71) indicating that the lower the fecal bile-acid excretion the higher the serum cholesterol level Fecal cholesterol did not correlate with that in serum

Discussion

The biochemical basis of familial hypercholesterolemia is unknown. Synthesis of plasma cholesterol from labeled acetate has been reported to be normal (11 12) and the liver of hypercholesterolemic individuals shows the normal suppressive effect of dietary cholesterol on cholesterol synthesis (16) Furthermore, radioactive cholesterol disappears from the circulation at about the normal rate (3 11, 12) An infant with moderate triglyceridemia and hypercholesterolemia without any family history has been reported to lack the feedback response to dietary cholesterol (6)

The low excretion of fecal bile acids now found in members of the family with pure hypercholesterolemia (without triglyceridemia) appears to be the first metabolic disorder described in this disease Lindstedt and Ahrens (14) observed a low daily synthesis of cholic acid in their two hypercholesterolemic patients The low bile acid excretion *could well be due to a genetic defect* affecting enzyme(s) converting cholesterol to bile acids in the liver This abnormally low catabolism could result in a gradual accumulation of cholesterol within the body and finally in hypercholesterolemia Increased bile acid absorption from the intestine might also lead to low bile acid excretion into feces and, through a feedback mechanism, to a low conversion of cholesterol to bile acids (2) In man bile acid feeding inhibits both endogenous synthesis of cholesterol and the conversion of cholesterol to bile acids without any effect on serum cholesterol level (9) On the other hand this family may excrete a sizable portion of bile acids through route(s) other than the intestine, or bile acids may in part be degraded to derivatives not detectable in feces by the method used At any rate the negative correlation between fecal bile acids and serum cholesterol suggests that the low bile acid excretion, whatever its origin caused hypercholesterolemia in this particular family That additional mechanism(s) for hypercholesterolemia might exist is indicated by

our preliminary studies on other hypercholesterolemic persons, some of whom excreted normal amounts of bile acids into feces

The contribution of dietary unabsorbed cholesterol to fecal cholesterol could not be determined in the present study Thus a possibility exists that the ab sorption of cholesterol in hyperchol esterolemics was better than normal and that the neutral steroid fraction could contain relatively more endogenous chol esterol than in controls On the other hand, low bile acid excretion into feces would accord with a possibility already suggested by Hofman (13), that micellar bile acid concentration during intestinal absorption may also be low in hypercholesterolemia Consequently, absorption of lipids including dietary cholesterol may actually be subnormal, which implies that neutral steroid fraction in feces could contain a sizable portion of dietary cholesterol The lack of correlation between fecal cholesterol and bile acids in hypercholesterolemics could well be due to predominance of dietary sterols Ac cordingly overall excretion of endog enous cholesterol from the body of these patients may actually be reduced even more than indicated by the low bile-acid excretion A normal synthetic rate of endogenous cholesterol could under these circumstances rapidly result in an enormous accumulation of cholesterol within the body Therefore to avoid this gross retention, a new steady state should be reached at a lower level of synthesis A low negative sterol balance found in hypercholesterolemic subjects suggests that this type of adaptation had actually taken place

Summary

Sterol balance studies showed that members of a family with hypercholesterolemia excreted subnormal amounts of bile acids into feces Neutral steroid ex cretion was within the normal range This finding, and a negative correlation found between fecal bile acid output and serum cholesterol level of hyperchol esterolemics, suggest that a decreased catabolism of cholesterol probably due to a low conversion of cholesterol to bile acids in the liver, was in this particular family the ultimate reason of hyperchol esterolemia and hence also of manifest xanthomatosis

Acknowledgements

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Addendum

In a more recent paper Hellstrom & Lind stedt (Amer J Clin Nutr *18* 46 1966) ob served a subnormal turnover of cholic acid in three of four hypercholesterolemic patients

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A Review of 295 Patients with Duodenal Ulcer

A follow up study 12—14 years after admission

By

KAJ VISKUM

Selection of the treatment of choice for patients with duodenal ulcer is difficult Surgical treatment more often than medical treatment leads to permanent cure, but the price is, for a number of patients sequelae which are directly referable to the operation According to the Scandinavian literature operative treatment has been used increasingly in recent years for patients with duodenal ulcers Thus Krarup (10) found in 1944 that only 6 % of patients who originally had been medically treated for duodenal ulcer were later operated Malmros and Hiertonn (12) examining a similar material in 1947, found that 17 % of the patients had been operated In the present material 42 % of the patients have been operated It is therefore of interest to see if this increase in the number of operated patients has changed the prognosis and course of the disease It is the purpose of the present study to compare the prognosis and course of the disease for operated and non operated patients, and to attempt to set up indications for the type of treatment most fitting for the individual patient The material illustrates the prognosis for medical and surgical treatment by estimating, among other things frequency of dyspepsia, working capacity, and status as judged by laboratory tests The material consists of 295 patients with duodenal ulcer confirmed by X ray who were admitted to Medical Department C, Bispebjerg Hospital in 1951 and 1952 The patients have been followed up in 1964 and 1965 The interim between observations is thus 12—14 years

Submitted for publication May 29 1967

Material and methods

The study comprises all patients admitted in 1951 and 1952 to Medical Department C Bispebjerg Hospital for dyspepsia or hematemesis/melena and in whom duodenal ulcer was found by X ray examination either as a niche or as a characteristic deformity of the duodenal bulb Patients who had been previously operated for duodenal ulcer have

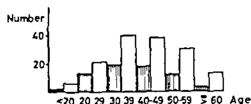


Fig. 1. Age at initial admission in 1951/52 for 208 men and 87 women (shaded columns = women).

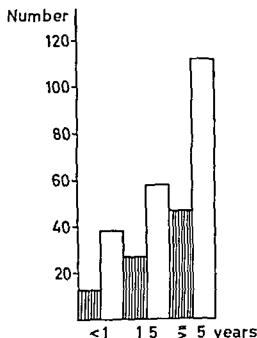


Fig. 2. Duration of symptoms before initial admission in 1951/52 for 208 men and 87 women (shaded columns = women).

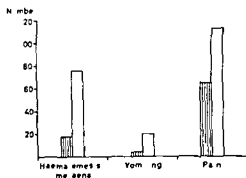


Fig. 3. Reasons for initial admission of 208 men and 87 women in 1951/52 (shaded columns = women).

been excluded (simple suture of ulcer perforation excepted). A total of 208 men and 87 women fulfilled these criteria.

The distribution according to age in 1951/52 is given in fig. 1 and reasons for admission and duration of dyspepsia prior to admission in figs. 2 and 3. A total of 111 (37 %) patients had already in 1951/52 been treated medically for duodenal ulcer one or more times.

Patients admitted in 1951/52 with massive hemorrhage were treated with egg-milk mixtures and oatmeal milk followed by a pureed diet whereas non-bleeding patients were fed through a duodenal tube for about three weeks after which a pureed diet was given. Antacids were given to almost all patients. The average duration of hospitalization was 25 days. At discharge a bland diet was recommended for half a year. None of the patients underwent an emergency operation for life-threatening hemorrhage but 30 (10 %) were transferred to the surgical unit for elective operation because of pylorostenosis, failure of medical treatment or a definite desire on the part of the patient. Among the remaining not primarily operated patients 236 (80 %) were completely symptom-free at discharge whereas 29 (10 %) still had dyspepsia.

Follow-up study

The patients were re-examined in the period between 1/12 1964 and 1/7 1965. All 295 patients were relocated. In the interim between 1951/52 and 1964/65 66 patients had died and their fate was determined through hospital records and information obtained from general practitioners. Of the remaining 229 patients 177 were examined by the author in the department's out-patient clinic or at their homes and 40 who had left Copenhagen or did not want personal contact with the investigator answered a written inquiry. Only 12 patients (six operated and six non-operated) did not wish to participate in the study. The cooperating patients were questioned about working capacity, symptoms of ulcer disease, diet habits and hospitalization. The operated patients were in

addition questioned about post prandial symptoms their personal evaluation of the result of the operation and its influence on their working capacity

Hemoglobin percentage and concentration of serum iron transferrin serum B12 serum calcium serum phosphate and alkaline phosphatase were determined in 170 patients Hemoglobin was determined according to Zijlstra and van Kampen (20, 21), serum iron and transferrin according to Young and Yocelin (19) serum B_{12} by micro biological techniques (6) serum calcium by flame photometry (4) serum phosphate ac cording to King (9) and alkaline phos phatase according to Kind and King (8)

Weights were determined for all patients at admission in 1951/52 and for 196 patients in 1964/65 when 162 were weighed in the out patient clinic and 34 stated their weight in the written inquiry Deviations from normal weight have been calculated using the normal weight tables of the Hafnia Insurance Company (16)

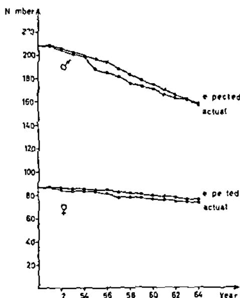


Fig 4 Expected and actual survival for 208 men and 87 women in the Copenhagen area

Results

In the interim 1951/52—1964 65, 95 men and 30 women (a total of 42 %) of the original 295 patients had been operated whereas 113 men and 57 women (a total of 58 %) had not been operated

Mortality

In the interim, 56 men and 14 women died On the basis of statistical information on the death rate in the Greater Copenhagen Area between 1950 and 1960 (18), the expected survival for the patients comprised in this study is calculated and compared with the actual survival (fig 4) The two curves do not differ significantly from each other with the exception of the years 1955 and 1956 during which the operated men in the material exhibited a significantly higher death rate ($p < 0.05$) The total number of deaths and the number of deaths from ulcer disease do not differ significantly in the operated and non operated patients A total of 4 % of the patients died in the interim from 1951/52 to 1964 65 from ulcer disease (0 3 % per observation year) The ex pectedly higher death rate from ulcer disease excepted no significant differ ence was found with respect to the cause of death between the general population and the present material

Comparison of operated and non-operated patients

The records from 1951 52 have been re viewed with respect to duration of dyspepsia prior admissions because of duodenal ulcer previous episodes of melena/hematemesis as well as average weight and age at admission (table I) Patients who were later operated had

TABLE I Admissions to medical ward for duodenal ulcer episodes of massive gastrointestinal hemorrhage and average durat on of symptoms prior to admission in 1951/52 average age and deviation from normal weight for 295 patients at admission in 1951/52 Comparison between 125 patients operated between 1951/52 and 1964/65 and 170 patients not operated

	No of adm	No of episodes of hem	Average duration of symptoms (yrs)	Average age (yrs)	Average deviation from normal weight (kg)
295 patients	151	152	8 9	46	—7 2
125 patients operated between 1951/52—1964/65	79	70	9 4	44	—7 5
170 patients not operated between 1951/52—1964/65	72	82	8 6	48	—7 1

TABLE II Review of 295 patients 12—14 years after initial admission in 1951/52

	Operated	Non operated	Total
	125 (42%)	170 (58%)	295
	(% of 125)	(% of 170)	(% of 295)
Deaths in observation period from ulcer d sease	7 (6 %)	4 (2 %)	11 (4 %)
Deaths in observation period from causes other than ulcer disease	17 (13 %)	38 (22 %)	55 (19 %)
Refused follow up	6 (5 %)	6 (4 %)	12 (4 %)
Clinical follow up	95 (76 %)	122 (72 %)	217 (73 %)
Cl nical status at follow up	(% of 95)	(% of 122)	(% of 217)
Symptom free	42 (44 %)	34 (28 %)	78 (36 %)
Mild symptoms	41 (43 %)	30 (24 %)	71 (33 %)
Severe symptoms	6 (6 5 %)	40 (33 %)	46 (21 %)
Disabled by ulcer disease	*6 (6 5 %)*	*18 (15 %)*	*22 (10 %)*

undergone significantly more medical ulcer treatments than those not operated ($0.02 < p < 0.01$) whereas the other differences between the two groups were not statistically significant

Review of the non operated patients

Of the 295 patients comprising this study 170 (58 %) had not been operated (table II) In the interim 42 (24 %) had died four (2 %) from ulcer disease (all in connection with hema temesis melena) Six patients did not wish to participate in the study Of the remaining 122 patients 34 (28 %) had for at least five years been completely symptom free and 30 (24 %) had only experienced slight intermittent dyspepsia

which did not cause working incapacity Fifty-eight (48 %) had more severe dyspepsia, which frequently or constantly required the use of a diet and or antacids Of these 58 patients, 18 (15 %) had within the last five years been substantially absent from work because of ulcer disease

Review of operated patients

Of the 295 patients comprising the study 125 (42 %) had been operated (table II) In the interim 24 (19 %) had died, seven (6 %) from ulcer disease (five in connection with operation, one from hematemesis and one from ileus caused by adhesions) Six patients did not wish to participate in the study Of the remaining 95 patients 42 (44 %) were completely symptom free and 41 (43 %) were only slightly disturbed by post prandial symptoms (36) or recurrence of ulcer dyspepsia (5) Only 12 (13 %) had severe post prandial symptoms (7) or more pronounced recurrence of ulcer dyspepsia (5) These symptoms had for six (6 5 %) of these 12 patients resulted in substantial absence from work in the last five years

Post prandial symptoms

Post prandial symptoms have been evaluated according to Meurling s scale (13) (table III) Symptom free pc 0, latent symptoms (pc), mild symptoms pc 1 and severe symptoms pc 2 and 3 In the clinical evaluation (table II) patients with pc 2 and 3 were included in the group with severe symptoms those with pc 1 and (pc) in the group with mild symptoms and those with pc 0 in the symptom free group unless symptoms of recurring ulcer disease justified other placement

TABLE III Incidence of postprandial symptoms in 95 operated patients (postprandial symptoms graded according to Meurling (13)

Grade	No
Pc 0	50 (53 %)
(Pc)	22 (23 %)
Pc 1	16 (17 %)
Pc 2	3 (3 %)
Pc 3	4 (4 %)

Symptoms of recurring ulcer

Among the 95 examined operated patients ten (10 %) had symptoms of ulcer recurrence three (3 %) as melena/hematemesis

The patients personal evaluation of the results of the operation

Among the 95 examined patients, 86 (91 %) were completely satisfied with the results of the operation and had the opinion that their working capacity had increased after the operation The results were not completely satisfactory for five (5 %) but these patients too, considered that their working capacity had increased after operation Only four (4 %) considered that their condition was poorer after the operation

Type of operation indications for surgery and age at operation

Half of the surgically treated patients were operated before the end of the year 1953 thereafter ca five patients were operated annually In 25 cases surgery was considered absolutely neces

TABLE IV Type of operative procedure and indications for surgery for 125 operated patients (operated 133 times)

Indications for operation	Exploratory laparotomy	Segmental resection	Billroth I	Billroth II	Pyloroplasty or gastroenteroanastomosis + vagotomy	Gastroenteroanastomosis	Total
Acute operation for massive hemorrhage	0	0	1	4	0	0	5
Suspicion of cancer	0	0	5	0	0	0	5
Pylorostenosis	0	0	0	8	1	6	15
Recurrent hemorrhage	*2*	*0*	*1*	*5*	*1*	*0*	*9*
Recurrence of symptoms after medical treatment	9	4	4	73	2	7	99
Total	11	4	11	90	4	13	133

TABLE V Age at operation for 125 operated patients

Age	No
<20	2
20—29	15
30—39	25
40—49	32
50—59	31
>60	28

sary 15 cases of polyrostenosis, five cases of life threatening hemorrhage and five cases of suspected malignancy in patients who developed concomitant stomach ulcer (in no case was malignancy found at operation (table IV) In the rest of the cases indication for surgery was relative, and several causes were involved in each case, reduced working capacity and recurrent episodes of melena/hematemesis were the most prominent The age at operation is given in table V The 125 patients have been operated a total of 133 times The cause for reoperation was recurrence of dyspepsia, most often following exploratory laparotomy Of the 133 operations 90 were uncomplicated, 38 had non lethal complications (pneumonia, phlebitis, lung emboli, wound infection, etc) and five patients died as a result of the operation (two from coronary occlusion, two from rupture of the anastomosis and one from sepsis)

Relation of course of disease to duration of symptoms

At admission in 1951/52 there was no statistically significant difference with respect to the duration of symptoms for non-operated patients as compared to patients who were subsequently operated Among the 122 examined non operated patients no relation was found between duration of symptoms and clinical status at follow up (table VI)

Occurrence of melena/hematemesis and ulcer perforation

The number of episodes of melena/hematemesis and ulcer perforation during the course of the disease have been calculated per 100 patient years for 1) non operated patients 2) operated patients before operation and 3) operated patients after operation (table VII) The occurrence of melena/hematemesis and perforation was greater among patients who were later operated than among those who were not operated After operation the frequency of hematemesis/melena as well as perforation was reduced considerably

Admissions to psychiatric wards

The number of admissions to psychiatric wards during the course of the disease has been calculated per 100 patient years for the same groups as mentioned above (table VIII) Group 3 had significantly more admissions than the first two groups ($0.02 < p < 0.01$) The reasons for admissions (mainly alcoholismus chronicus, psychopathia, neurosis and tentamen suicidii) did not differ for the three groups

TABLE VI Duration of symptoms prior to admission in 1951/52 compared to clinical status at follow up in 1964/65 for 122 non operated patients

Duration (yrs)	Symptom free	Mild symptoms	Severe symptoms
<1	7	4	9
1—5	11	11	19
>5	16	15	30
	34	30	58

Objective findings

At the follow up in 1964/65 the non operated patients, men as well as women, had come closest to normal weight (table IX), whereas the operated patients were nearly as much underweight as at admission in 1951/52

The mean values for hemoglobin serum iron transferrin serum B_1, serum phosphate, serum calcium and alkaline phosphatase are given in table X Only the mean values for hemoglobin and serum B_{12} for operated and non operated men differed significantly, being lowest for the operated It must be remembered, however, that 28 operated patients as opposed to only 6 non

TABLE VII Incidence of massive gastrointestinal hemorrhage and ulcer perforation in three subgroups viz non operated patients and operated patients in pre and post operative period

	Observation period (yrs)	No of massive gastrointestinal hemorrhages	Massive hem /100 obs yrs	No of ulcer perforations	Ulcer perforations/100 obs yrs
170 non-operated patients	3 388	110	3 2	4	0 1
125 operated patients in pre-operative period	1 536	114	7 4	20	1 3
125 operated patients in post-operative period	067	8	0 7	0	0 0

TABLE VIII Admissions to psychiatric wards for non-operated patients and operated patients in pre and post-operative period

	Obs yrs	No of admissions	Admissions /100 obs yrs
170 non-operated patients	3 388	50	1 5
125 operated patients in pre operative period	1 536	22	1 4
125 operated patients in post operative period	1 067	33	3 1

operated were being treated at the time of the follow up with iron, and five operated as opposed to no non operated patients were being treated with B_{12}. Of the 74 operated patients 22 were anemic as compared to seven of 96 non-operated patients ($p < 0.01$) (table XI) Likewise subnormal values for serum iron and serum B_{12} were more frequently found among the operated patients These differences are significant (for iron $0.05 < p < 0.02$, for B_{12} $p < 0.01$) Values of serum B_{12} lower than 125 picogram/ml were never found earlier than five years after operation

TABLE IX Average deviation from normal weight (kg) for operated and non operated patients at initial admission in 1951/52 and at follow up in 1964/65 (normal weight according to Hafnia s weight tables (16)) (± SE)

	Men		Women	
	Operated 66	Non operated 70	Operated 22	Non operated 38
1951/52	−8 1 ± 0 9	−6 5 ± 0 9	−5 6 ± 2 5	−8 3 ± 1 5
1964/65	−5 8 ± 1 2	−0 3 ± 1 1	−7 9 ± 2 3	−4 6 ± 1 8

TABLE X Mean values for hemoglobin serum iron total iron binding capacity serum B_{12} serum calcium serum phosphate and alkaline phosphatase for operated and non operated patients at follow up (± SE)

	Men		Women	
	Non operated 63	Operated 56	Non-operated 33	Operated 18
Hemoglobin g %	14 3 ± 0 1	13 4 ± 0 2	13 2 ± 0 1	12 9 ± 0 4
Serum iron μg %	106 ± 4	101 ± 7	95 ± 6	99 ± 11
TIBC, μg %	325 ± 6	346 ± 7	332 ± 7	324 ± 16
Serum B_{12} picog/ml	416 ± 20	295 ± 18	378 ± 28	385 ± 49
Serum calcium mg %	10 6 ± 0 1	10 4 ± 0 1	10 7 ± 0 1	10 4 ± 0 2
Serum phosphate mg %	3 3 ± 0 1	3 5 ± 0 1	3 6 ± 0 1	3 4 ± 0 1
Alkaline phosphatase KA units	7 0 ± 0 3	6 8 ± 0 3	6 6 ± 0 3	6 9 ± 0 7

TABLE XI Operated and non-operated patients with abnormally low levels of hemoglobin serum iron and serum B_{12} at follow up

	Men		Women	
	Operated	Non operated	Operated	Non-operated
	56	63	18	33
Hemoglobin				
<13 g % men <12 g % women	17	5	5	2
Serum iron				
<55 µg %	8	2	2	2
Serum B_{12}				
<175 picog/ml	7	1	1	1

Discussion

The survival of the patients in this study differs only very little from the expected survival and the causes of death, apart from a higher mortality from ulcer disease are the same as expected

Krarup (10) and Malmros and Hiertonn (12) found in similar studies a mortality from ulcer disease of respectively 0 5 % and 0 4 % per observation year whereas this study showed a mortality of 0 3 % In the two previous studies pylorostenosis and ulcer perforation caused 35—50 % of the deaths whereas no person in this study died from these causes In spite of the much higher frequency of operation in this study, the number of deaths resulting from operation amounted to a proportion of the total material no higher than in the previous two studies Likewise death from melena hematemesis represented the same percent of the total material as in the two studies mentioned above

Krarup and Malmros and Hiertonn showed that 80—90 % of patients treated medically for duodenal ulcer become symptom free during the treatment period. The treatment given in the present study was rather peculiar, and because of its unpleasant nature it is hardly used any longer Still, the number of patients who were symptom free after this treatment amounted to 80 % of the total, a fact which seems to imply that the actual type of medical treatment given is of little if any importance Krarup (10) and Malmros and Hiertonn (12) showed too that the long term results of medical treatment were disappointing as only ca 20 % of the patients remained symptom free, while 30 % had recurrent mild and 50 % recurrent severe symptoms Furthermore Quigstad and Romcke (15) demonstrated that the number of symptom free patients declines quickly with extended observation time The above mentioned authors therefore concluded that the long term results of medical treatment were unsatisfactory and that an increasing number of patients ought to be operated

The results of surgical treatment have been reviewed by among others Blixen Krone Møller (1) and Danielsen (2) who found that the long term results of surgical treatment were materially better than those of medical treatment Danielsen (2) reports that 76 % of the patients had been cured but he bases his results on the patients personal evalua

tion of the result of the operation, whereas Blixenkrone Møller, using an objective evaluation of the patient's status, found that only 41 % were completely free of symtoms. The rest of the patients had varying degrees of symptoms, e.g. 14 % had pronounced postprandial symptoms.

The present study cannot be compared with Krarup's (10) and Malmros and Hiertonn's studies (12), as these authors reviewed the results of medical treatment for duodenal ulcer, and therefore they classified all operated patients as belonging to the group with severe symptoms, disregarding the final outcome of the surgical treatment. The present results suggest, however, that the number of totally symptom free patients is considerably greater after recent years' combined medical/surgical treatment, just as the number of patients with severe symptoms has been markedly reduced. This change of the prognosis and the course of the disease is due to the improved results of surgical treatment, as present results of medical management differ only insignificantly from the results in the two above mentioned studies.

In the pre operative period the subsequently operated patients had significantly more episodes of melena, hematemesis and perforation than the non operated patients, and they had been admitted more frequently for medical treatment for their duodenal ulcers. Postoperatively the great majority of the patients had regained full working capacity, none had had perforations and only very few melena/hematemesis. These results probably explain why the majority of these patients were satisfied with the result of the operation in spite of sequelae.

The clinical status at follow up was best in the group of operated patients, as only 13 % of these, as opposed to 48 % of the non operated patients, could be classified as poor. This is especially remarkable in view of the fact that patients who received medical treatment exclusively had originally had on the average, milder symptoms than those who were subsequently operated.

The frequency with which postprandial symptoms were found is similar to Meurling's (13) findings. Likewise the frequencies for ulcer recurrence, subnormal serum levels of vitamin B_{12} and anemia were found to agree with earlier investigations of partially gastrectomized patients (17). It has been claimed that osteomalacia is more frequent among partially gastrectomized than among non operated patients with duodenal ulcer (5), but the frequency with which osteomalacia occurs is disputed, as is the significance of abnormal alkaline phosphatase and serum calcium values in this condition (11). Disturbances in calcium resorption after partial gastrectomy are most often seen among older patients 10—15 years after operation. Thus the frequency with which osteomalacia is found in a given study is dependent upon the percentage of the total material which these elderly patients represent. In this study the mean values for alkaline phosphatase, serum calcium and serum phosphate for operated and non operated patients did not differ significantly. A possible explanation of this fact is that only a small part of the patients were more than 70 years

of age and that the interim following operation was at most 14 years, and for the majority of the patients considerably less Psychiatric disturbances, especially neurosis, have been reported as beginning concomitantly with or following partial gastrectomy (7) This was confirmed in the present study in which there were twice as many admissions of operated patients to psychiatric wards per 100 patient years after operation as before

The results indicate that medical treatment for duodenal ulcer should be reserved for patients with initial symptoms and mild recurrent symptoms whereas patients with working incapacity or recurrent massive hemorrhage or perforation should be operated as early as possible This is especially true as the severity of symptoms is of major importance with respect to the choice of treatment, less weight should be placed upon duration of symptoms than has previously been done The operative mortality the complication rate and the frequency of sequelae after operation are not in opposition to this viewpoint

Summary

Two hundred ninety five patients admitted to the Medical Ward with radiologically confirmed duodenal ulcer in 1951/52 have been followed up after 12—14 years Clinical information has been obtained concerning 96 % of the patients In the interim 42 % of the patients had been operated whereas 58 % had received medical treatment exclusively The results of the last 14 years of combined medical surgical treatment of duodenal ulcer are better than the results from the previous years during which the patients received mainly medical treatment More patients are now completely symptom free and fewer have severe symptoms Death due to ulcer perforation and pylorostenosis did not occur in this study whereas the frequency of death in connection with operation and hemorrhage is unchanged as compared to earlier studies The survival rate for the patients in this study does not in practise differ from the expected survival rate for the population-at large There was no significant difference between the number of deaths among operated and non-operated patients Patients subsequently operated had had, as a whole, more severe symptoms than patients who received solely medical treatment In spite of this fact the clinical status at follow up was best for the operated patients The surgical treatment had reduced the frequency of melena hematemesis considerably and ulcer perforation did not occur after operation Half of the operated patients had post prandial symptoms but only a few to a severe degree Among the operated patients 30 % were anemic and 20 % had subnormal serum levels of vitamin B_{12} or were under treatment with vitamin B_{12} At the follow up the operated patients were more underweight than the non-operated patients The surgically treated patients were on the average admitted twice as frequently to psychiatric wards in the years following operation as compared with the years prior to operation The results indicate that patients with severe symptoms of

duodenal ulcer, regardless of the duration of the symptoms, will benefit from an operation Approximately half of the patients in this study belong to this group In the long run probably even more operated patients will develop anemia and lack of vitamin B_{12} Likewise the frequency of osteomalacia will increase This argues in favor of frequent control of the operated patients as well as prophylactic treatment with iron and vitamin B_{12}

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Worm Cure Without Tears

The effect of niclosamide on taeniasis saginata in man

By

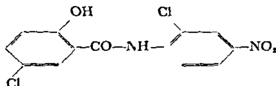
JORN DITZEL and MICHAEL SCHWARTZ

Taeniasis saginata is the most common variety of tapeworm in this country (Denmark) Whilst the actual symptoms it will evoke are usually mild, it is a very disagreeable experience for many to observe the independently moving proglottids either during or between defaecation

The treatment of choice in most Danish hospitals is the unpleasant experience of 'filix cure On admission the patient is given black coffee and pickled herring followed next morning by a water enema and the administration of Oleoresina filicis malis (WHO) The latter may cause vomiting A little later he receives a cathartic dose of magnesium sulfate and finally at about 2 p m another water enema Treatment always requires bed rest, and in some hospitals a fat free diet for several days before admission is part of the regime employed The use of Oleoresina filicis malis is not completely without risk Even therapeutic doses can cause poisoning with xanthopsia temporary blindness, gastroenteritis and abdominal pain muscle cramps and subsequent tonic convulsions, respiratory failure and cardiac arrythmias It is contra indicated in heart, liver and kidney disease and throughout pregnancy

Yomesan

It is therefore of some interest that the substance niclosamide (N (2 chloro-4 nitrophenyl) 5 chlorosalicylamide



has been shown to act as a simple harmless and successful therapy in all cestodian infections of man being effective against taenia saginata taenia solium, diphyllobotrium latum and hymenolepsis nana It was tested on a large scale in trials on animals by Gonnert and Schraufstatter (1) and also subjected to

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extensive toxicological studies by Hecht and Gloxhuber (2) in 1960 As the test-drug Cestocid Bayer 2353 it underwent clinical trials in Germany, Finland and Africa (3 4, 6)

Marketed now as Yomesan the substance itself is a tasteless odourless, yellow white powder almost insoluble in water which acts by interfering with the metabolism of the scolex and neighbouring proglottids as soon it reaches the organism in the gastrointestinal tract The prominent suckers are forced to release their grip and the organism in its entirety is dislodged at the following defaecation

Its use in practice is simple four tablets of Yomesan (500 mg) are given at a small breakfast to be chewed well and washed down with a little water and two hours later a saluretic laxative such as magnesium sulfate (20 g) is administered An adult dose is given to children if they are aged six years or more but younger patients receive two tablets and if the child is younger than two years one tablet only is required

The purge may be omitted but in taenia solium infections extremely unusual in Denmark a laxative must always follow the use of niclosamide as without it there is a risk that the digestion of dead segments could cause liberation of viable ova into the gut lumen and subsequent cysticercosis

As far as it is known there are no contraindications to the use of niclosamide no side effects are known and it is of considerable interest that it may be employed in pregnancy after the first trimester Any kind of medication is undesirable in the first trimester and niclosamide is no exception

Own investigations

During a nine month period we have employed niclosamide in the treatment of 48 cases of infection with taeniasis saginata

The regime used was that as outlined above In all cases the worm was removed successfully A follow up study, three months after treatment, showed no recurrence The combination of gentleness and effectiveness has made the treatment very popular In no patients have any side effects of any description been seen

Our experience which has confirmed the optimum efficiency, safety and simplicity of niclosamide make us recommend it as a standard treatment and we feel also that admission for 'wormcure should be a thing of the past as the home doctor can easily administer the remedy

Summary

Forty-eight patients with taeniasis saginata have been treated with the vermicide Yomesan niclosamide (N (2 -chloro-4 nitrophenyl)-5 chlorosalicylamide) In all cases the worm was removed successfully The treatment is very effective totally harmless and so simple (four tablets) that admission to the hospital is unnecessary

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Atrial Fibrillation and Flutter Treated with Synchronized DC Shock

A study on immediate and long term results

By

B Wikland, O Edhag and H Eliasch

Conversion of atrial arrhythmias to sinus rhythm is for several reasons desirable Atrial fibrillation carries considerable risk of systemic and pulmonary embolism especially in rheumatic heart disease (3, 13) In fibrillation cardiac output in response to exercise is unsatisfactory (15) Digitalis and, more so quinidine have traditionally been the main tools for the control of atrial arrhythmias Quinidine has to be given in high dosage and the individual dose response to this drug is variable Serious arrhythmias and even sudden death may occur (9 11 14) Manifestations of quinidine toxicity have been reported to occur in up to 35 % of patients during attempts at conversion and sudden death in 2—3 % (11, 14) Moreover, the conversion attempt, during which the patient needs careful supervision may be time consuming

Since the introduction of the Lown technique of synchronized DC shock (7) for the termination of atrial arrhythmias this method has rapidly gained widespread use It is generally agreed that synchronized DC shock is a safe and effective method for restoring sinus rhythm (4 6 10) Follow up investigations however indicate a high rate of recurrence of atrial arrhythmia within a relatively short period of time (4 6 10) In the present study our experience with synchronized DC shock in atrial fibrillation and flutter is reported and possible clues to subsequent prognosis are discussed

Material

Seventy four unselected patients (52 males and 22 females aged 34 76 years—mean 56 years) were treated with synchronized DC shock A total of 110 attempts were made during which 206 shocks were deliv

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TABLE I Clinical diagnosis and duration of sinus rhythm

	n pts before DC shock	Sinus rhythm after 2 months		12 months	
		n	%	n	%
Mitral valvuar disease (not op)	17	8/17	47	4/16	25
Mitral valvular disease (op)	10	4/10	40	1/10	10
CHD with or without systemic arterial hypertension	17	9/16	56	5/12	42
Miscellaneous	30	15/29	53	5/22	23

ered Sixty six patients had atrial fibrillation and eight had atrial flutter

The patients were divided into four clinical groups as listed in table I 1) mitral valvular disease (not operated) 2) mitral valvular disease (operated) 3) coronary heart disease with or without systemic arterial hypertension and 4) miscellaneous conditions (such as I^{131} treated hyperthyroidism presumed alcoholic heart disease, operated and nonoperated atrial septal defect and myocarditis)

Methods

All patients were hospitalized and all received adequate anticoagulant therapy for 2—3 weeks before the conversion attempts All patients except two were given a maintenance dose of digitalis Patients with signs of congestive heart failure were treated with digitalis and diuretics prior to conversion attempts In each patient a chest X ray examination was done before the conversion attempt Electrolyte balance was assessed and corrected if necessary before conversion was attempted Duration of arrhythmia preceding DC shock was recorded as the period of time after the patient first experienced a definitely irregular heart rhythm If no such history was given the date when atrial arrhythmia was first diagnosed by a physician was recorded as the beginning of arrhythmia Duration of arrhythmia in patients with paroxysmal fibrillation or flutter was recorded as the time elapsed since the first spell of arrhythmia

The synchronized DC shocks were delivered by a Lown Cardioverter (American Optical Company) A four channel ink writing electrocardiograph (Mingograf® Elema Schonander) was used to record the electrocardiogram before and after every DC shock Before delivery of the synchronized DC shock all patients were anesthetized with a short acting barbiturate (Pentothal Sodium®) The energy level ranged from 100—400 Wsec and the first shock was usually of low energy (not exceeding 150 Wsec) Following successful conversion all patients were given if tolerated 1 2—1 6 g per day of a slow release quinidine preparation (Kinidin Duretter®), half the daily dose given in the morning the other half in the evening Four patients who did not tolerate quinidine were given procaine amide (Pronestyl®) 0 5 g four times daily after DC conversion

Results

Sinus rhythm persisted in 60 out of 74 patients (81 %) for 24 hours after DC shock Two months later, 36 out of 72 patients (50 %) still had sinus rhythm and two patients had been lost to follow up At the end of the follow up period (one year after DC shock) 15 out of 60 patients (25 %) remained in sinus

TABLE II Duration of arrhythmia preceding DC shock vs persistence of sinus rhythm

Follow up period months	< 1 year		1—3 years		> 3 years	
	n	%	n	%	n	%
2	18/34	53	4/10	40	15/28	54
12	10/29	35	1/17	14	4/25	16

TABLE III Heart volume ml/m² BSA in relation to subsequent rhythm

Follow up period months	—449		450—649		650—	
	n	%	n	%	n	%
2	7/10	70	25/43	58	5/19	26
12	2/10	20	10/36	28	3/18	17

rhythm as seen in fig 1 The remaining 12 patients were not included since the follow up period was less than one year

It is seen from table I that the percentage of patients remaining in sinus rhythm after intervals of two months and one year was lowest in the group of operated mitral valvular disease (40 % and 10 % respectively) and highest in the group of coronary heart disease with or without systemic arterial hypertension (56 % and 42 % respectively)

The relationship between the duration of atrial fibrillation or flutter preceding DC shock, on the one hand, and heart rhythm two months and one year later on the other, is shown in table II It is seen that the percentage of patients in sinus rhythm after two months did not differ in the two groups having a duration of arrhythmia less than one year and more than three years At the one year follow up these two groups differed the group with arrhythmia for less than one year had sinus rhythm in a higher percentage (35 %) than the group with arrhythmia of more than three years duration (16 %)

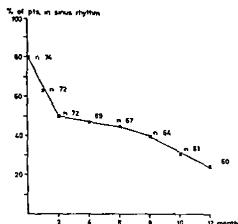


Fig 1 Duration of sinus rhythm after DC shock

Heart volume and percentage of patients remaining in sinus rhythm two months and one year after conversion attempt is shown in table III After two months, increased heart size was associated with an almost proportionate fall in percentage of patients with sinus rhythm At the one year follow up this relationship was not found

One or more attempts at quinidine conversion were made in 27 cases before DC shock Of these, 11 (41 %) resulted in conversion to sinus rhythm and later relapsed into atrial arrhythmia Two months after DC conversion attempts seven of these 11 patients (64 %) remained in sinus rhythm Of the 16 patients with previous quinidine failure nine (56 %) remained in sinus rhythm two months after DC conversion attempt

Sinus rhythm persisted for at least 24 hours after DC shock following 29 conversions at energy levels not exceeding 150 Wsec Two months later 17 (59 %) of these patients remained in sinus rhythm If the arrhythmias were not terminated at low energy levels, Wsec settings were gradually increased Twenty eight attempts were made at 300 Wsec or more seven (25 %) of which yielded sinus rhythm persisting two months later

Twenty two patients have undergone two or more attempts at DC conversion After the initial attempts sinus rhythm was still present at two months in 12 (55 %) patients Forty nine patients underwent only one conversion attempt, of whom 23 (47 %) still had sinus rhythm after two months

Of the 66 patients with atrial fibrillation, 56 (85 %) had F waves of 0 1 mV or more in lead V_1 Two months after DC shock four (40 %) of the ten patients without F waves of this magnitude remained in sinus rhythm One of the eight patients with atrial flutter was lost to follow up after two weeks Of the remaining seven patients five had sinus rhythm two months after DC shock

Quinidine at 1 2 g/day, the minimum dosage required for adequate plasma levels (2), has been tolerated by 34 patients, of whom 21 (62 %) had sinus rhythm two months after DC shock Thirteen (54 %) of 24 patients without satisfactory quinidine maintenance remained in sinus rhythm two months after their conversion attempts At the one year follow up seven (25 %) of 28 patients observed with adequate quinidine maintenance still had sinus rhythm, whereas eight (44 %) of 18 patients without satisfactory quinidine maintenance therapy remained in sinus rhythm Of the four patients given 0 5 g procaine amide four times daily none remained in sinus rhythm after two months

No complications have been observed during or immediately after DC conversion One patient had signs of mesenteric vascular occlusion on the fourth day after conversion in spite of the presence of sinus rhythm and adequate anticoagulant therapy

Case reports

Case 1

A 45 year old woman was operated in 1959 for mitral stenosis after which moderate mitral incompetence developed Atrial fibrillation was first noticed in early 1964

accompanied by moderate exertional dyspnea At that time heart volume was 560 ml/m BSA and serum electrolytes normal Conversion to sinus rhythm was obtained on the initial attempt at 150 Wsec and she was maintained on quinidine (Kinidin Duretter® 1 6 g/day) and digoxin (0 25 mg/day) for 13 months before relapse into atrial fibrillation No change had occurred in heart size or electrolyte status A conversion attempt one week later failed despite three DC shocks at 400 Wsec

Case 2

A 36-year old man was in good health until the summer of 1964 when he developed diarrhea and fever followed a month later by palpitations and breathlessness Atrial fibrillation was diagnosed and an attempt at conversion with quinidine failed When the patient was referred to Serafimer Hospital six months later there was no evidence of congenital or rheumatic heart disease hyperthyroidism or cardiac enlargement B P was 120/65 mm Hg and serum electrolytes normal in March 1965 when conversion was attempted with successive attempts at 150 250 and 350 Wsec before sinus rhythm was established after a second shock at 350 Wsec Sinus rhythm was still present at the 17 months' follow up

Case 3

A 60 year old man who was previously in good health developed atrial flutter in 1964 This was converted to sinus rhythm with quinidine but reverted to flutter after only two weeks In April 1966 after two years without conversion attempts he was admitted for DC conversion B P was 120/75 mm Hg, cardiac configuration was normal (420 ml/m² BSA) there was no evidence of rheumatic or congenital heart disease and electrolytes were normal on the conversion day Three DC shocks were administered at 150 200 and 350 Wsec without effect One week later without change in medication, another attempt was made at 300 Wsec and sinus rhythm was obtained which remained at a six month follow up

Discussion

The short and longer term results agree well with those of others (4 6, 10) As described, anticoagulant therapy and maintenance quinidine, when tolerated, have been given throughout the study and this is believed to be a common policy in Sweden The value of anticoagulant therapy before conversion attempt has been questioned by several workers Killip (5) found a comparable incidence of embolism whether anticoagulants were given or not Lown (8) has not established precise criteria for giving anticoagulants Halmos (4) has published a study of 175 patients treated with DC shock, none of whom received anticoagulant therapy prior to conversion attempt and only one developed embolism Anticoagulant therapy, per se is not without risk although no bleeding complications were encountered in the present study It seems justified to question the necessity of this treatment before conversion attempt

The need of an antiarrhythmic maintenance treatment with quinidine after successful DC conversion has been stressed by Korsgren et al (6) In contrast, Halmos (4) found no difference in sinus rhythm stability between three groups of patients receiving respectively 0 3 g quinidine sulphate four times daily an effervescent potassium preparation and no antiarrhythmic therapy Our results do not lend support to the concept that quinidine exerts a stabilizing effect At the moment it is not possible to select patients for DC conversion by clinical

diagnosis Previous successful quinidine conversion provides a clue to future sinus rhythm stability, but quinidine failure does not justify exclusion of at least one attempt at DC conversion Quinidine conversion is steadily giving way to DC conversion, a fact that will make this point less important in the future

It is known from quinidine conversions that a long standing arrhythmia is more difficult to convert and maintain in sinus rhythm than one of short duration That this holds true also for DC conversion has been demonstrated by Korsgren et al (6) and the same tendency was observed in the present study

The fact that a large heart volume per se, does not exert any consistently unfavourable influence on sinus rhythm stability has been reported by Korsgren et al (6) and the situation at the one year follow up in our study gives support to this observation On the other hand two months after conversion there was an almost proportionate decrease of the percentage of patients remaining in sinus rhythm with increasing heart volume leaving relatively few patients with large hearts for the one year follow up and making the figures for the longer period less reliable

None of Oram and Davies (10) patients requiring 400 Wsec converted to sinus rhythm and it has been customary to regard patients requiring high energy levels to be less stable in sinus rhythm if it can be established In this study there seems to be a varying individual sensitivity to the energy of the DC shock some individuals requiring higher energy levels for conversion One case requiring high energy was described above (case 2) The fact that seven out of 28 attempts at 300 Wsec or more, following failure at lower energy settings, have given sinus rhythm persisting two months or more justifies at least one high energy attempt

Twenty two patients underwent more than one DC conversion attempt The reason for further attempts was most commonly late relapse into atrial arrhythmia after a successful first attempt The prognosis for a second attempt is not always good, as is illustrated above (case 1) On the other hand, resistance to even high energy shocks may not exclude subsequent successful conversion and stable sinus rhythm, as shown in case 3

It has been reported (1) that quinidine conversion is more difficult in patients with small or absent F waves in the ECG Ischemic heart disease has been associated with small or absent F waves while larger F waves have been reported in rheumatic heart disease (12) Oram and Davies (10) and Halmos (4) had significantly better results with DC conversion when large (0 1 mV or more in lead V_1) F waves were present However, in our study with unselected patients F waves of 0 1 mV or more in lead V_1 are found in 85 % of patients with atrial fibrillation The remaining 15 % (ten patients) constitute too small a group to permit conclusions about the stability of sinus rhythm after conversion

Summary

Conversion of atrial arrhythmias by synchronized DC shock was found to be a safe and effective procedure The

greatest problem consisted in the maintenance of sinus rhythm once it was established The stabilizing effect of quinidine was uncertain, and quinidine itself often causes diarrhea and dizziness when given in adequate dosage (at least 1 2 g/day) At the moment it is not possible to predict the stability of sinus rhythm, if established, in the individual patient with atrial arrhythmia Clinical diagnosis and presence of F waves in the ECG lead V_1 do not, in our study, permit any conclusions about subsequent prognosis Previous successful quinidine conversion indicates a stable sinus rhythm after DC conversion, but quinidine failure cannot be regarded as a reason for withholding at least one attempt at DC conversion A large heart is an unfavourable prognostic factor, but the fact that synchronized DC shock is a safe and quick procedure justifies its liberal use if sinus rhythm could be of benefit to the patient In, for instance very old patients who have little demand of physical exercise and, therefore, little need for the increased cardiac output made possible by sinus rhythm, conversion of atrial arrhythmia seems of questionable value If the patient does not respond to low energy shocks at least one attempt at the 400 Wsec level appears justified In a given individual the response to DC shocks seems to be variable, which makes a second attempt reasonable in the face of an initial failure if the patient definitely would benefit from sinus rhythm

Acknowledgement

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Book review

Hormones and connective tissue By G Asboe-Hansen 431 pages D cr 103 — Munksgaard, Copenhagen 1966

This volume is the second on the same subject edited by Professor Asboe Hansen The first, Connective Tissue in Health and Disease, published in 1954, made a pioneering contribution to the field of connective tissue research Over the years an enormous amount of new information on this tissue has accumulated Its biochemistry has been studied by outstanding research groups, and there can be no doubt that connective tissue in its widest aspect will occupy a central place in medicine in the near future

Normally produced hormones play an important role in the normal and pathological physiology of the connective tissue since they exercise a constant control over the function of mesenchymal structures The present volume is intended to deal with this part of the field However, it extends into areas the connection of which with endocrinology and hormones is vague, unclear or non-existent arteriosclerosis, skin disease, inner ear function, surgical bleeding etc The chapters are written by people affiliated to the University of Copenhagen and engaged on the research in this field

This monograph, according to the editor, does not pretend to be a complete treatise on connective tissue endocrinology—and is far from being so It does not give the reader any overall view of the relations between hormones and mesenchymal tissue Its asset is that it presents articles on special areas of the field written by authors who have studied them for lengthy periods and are very well acquainted with the development in these areas In this way the volume presents, rather, pieces of a broad medical field than a view of the field as such

All those active in research on mesenchymal tissue will read this book with great benefit

Rolf Luft
Stockholm

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From the Departments of Medicine, Radiology and Surgery of the University Hospital Uppsala Sweden

Pheochromocytoma Diagnostic Features

By

Erik Ask Upmark, Folke Knutsson and Lars Thorén

Pheochromocytomas are rare tumours In the U S A their incidence has been estimated to be about 1,000 a year For Sweden a corresponding figure should be about 40 a year According to the last report on tumour incidence in Sweden (for 1961), ten such tumours were found It thus seems reasonable to assume that most instances have remained undetected This is because of their rarity and their protean clinical manifestations Yet if undiscovered these tumours are likely to end fatally (cerebral haemorrhage, pulmonary oedema cardiac infarction) It may, therefore, be useful to give a brief review of some diagnostic features to illustrate their variety

Material

Our material is limited to ten cases during the last 11 years In one case there were tumours in both adrenals so the total number of pheochromocytomas is 11 Five of our cases were males aged 16—50 years and five were females aged 33—77 years

Case 1

Woman aged 49 Three deliveries When aged 15 she had meningitis with fever and rigidity of neck, treated at home At age 40 she underwent cholecystectomy, following severe attacks of pain which were somewhat ameliorated after the operation

Since at least her early thirties she had migraine which was treated by various compounds including phenacetin resulting in damage to her kidneys A peculiar feature was her ability for several years to precipitate pains in the back of her head by extending the left arm upwards or by carrying heavy objects with her left hand

When aged 49 she had a subarachnoid haemorrhage for which she came to our department of medicine Angiography revealed an aneurysm at the start of the left a cerebri media This was operated upon One or two days afterwards aphasia and rightsided hemiplegia developed Further operation revealed that the aneurysm had been well treated but an intracerebral haemorrhage in the left temporal lobe had occurred This was evacuated but the patient died At autopsy a pheochromocytoma in the left suprarenal gland was found which was undoubtedly responsible for the terminal haemorrhage The kidneys were severely affected by the previous pyelonephritis (phenacetin kidneys)

Case 2

Man aged 19 The patient s mother is said to have high B P When aged 17 a routine

Submitted for publication May 10 1967



a



b

Fig 1 a—b Case 2 Angiogram showing a tumour of the right suprarenal gland

Health examination revealed a B P of 150 80 and one year later this was 160 100 At age [illegible] he had a B P of 185/95 with somewhat attenuated retinal arteries Determination of catecholamines in 24 hr urines showed noradrenaline 108 8 later 230) μg and adrenaline [illegible] later 5 7) μg Abdominal aortography revealed the presence of a tumour at the upper pole of the right kidney (fig 1 a—b This was removed at operation Hulten and found to be a pheochromocytoma with a weight of 20 g

Case 3

Woman aged [illegible] This patient was admitted to the department of medicine in a critical condition, following a cardiac infarction which eventually caused a perforation of the interventricular septum of the heart near the apex The patient died and autopsy confirmed the cardiac observations but revealed also a plum sized pheochromocytoma of the left suprarenal gland (weight 40 g

Case 4

Man aged 47 One younger brother died from cardiac infarction when aged 25 The patient was a blacksmith working in a foundry at temperatures of 60—70 C When aged 17 he started to get attacks of anxiety and oppression in the heart region when he started work after his dinner If he rested for a short while the discomfort passed by but it troubled him until 20 years of age

At age 36 he had the same symptoms this time not after meals but after he had gone to bed the symptoms were severe with pain in the heart region as well as in both arms In addition he had an intense headache, vomiting (bringing relief) and a pulsating noise behind both ears When such an attack subsided his neck was considerably swollen, having a circumference of 57 cm instead of the normal 39 cm He maintained that high B P had been frequently registered during the attacks on one occasion even above 350 The attacks could be precipitated when





a b

Fig 2 Case 4 The heart size at the time of removal of a pheochromocytoma was 580 ml m² (a) and 460 ml/m five years later (b)

straining at stool Heart size was found to be 580 ml/m² body surface and ECG confirmed hypertrophy of left ventricle B P was 240 130 and one month later 210/175 Urography normal Urine concentration test showed specific gravity of 1 025 Urine catecholamines were moderately increased h 74 and 88 µg noradrenaline in 24 hr urines Retroperitoneal insufflation of air revealed a rightsided tumour on the top of the kidney At operation (Hulten) a hen egg sized tumour was found between the kidney and the suprarenal This was removed and although it appeared rather more yellow than a pheochromocytoma histology showed it to be a typical tumour of this character

Subsequently his urine catecholamines were normal but the B P was still increased (190 130) The heart size five years after the operation had decreased to 460 ml/m (Fig 2 a—b)

Case 5

Man, aged 50 The patient was the fourth of eight children One sister died from diabetes and one brother from arterial hypertension For the last few years the patient had attacks or periods of dyspnoea, frequently combined with pulsating headaches If performing a Valsalva test as in most attacks the symptoms are apt to get worse Recently his visual acuity had been reduced

B P in the supine position was 215 135 and in the standing position 120 80 ECG showed left hypertrophy but the heart size was fairly normal The fundi of the eyes showed protrusion of the discs serpentine vessel haemorrhages and exudates Basal metabolic rate was —6 and on another occasion +23 % Fasting blood sugar was 200 mg% Catecholamines in the urine were increased Whilst oxygen insufflation in the retroperitoneal space yielded no definite result aortography revealed a vascularized tumour at the medial side of the right kidney This tumour was at least the size of a hen's egg At operation (Hulten) the tumour was found to be situated around the right renal artery and in order to remove it nephrectomy was necessary The tumour was obviously malignant and extended into the surroundings He died from anuria in spite of attempts at dialysis

Case 6

Woman aged 59 The patient's father died from apoplexy Her only sister has headaches Since the age of 15 the patient had migraine with headaches and vomiting

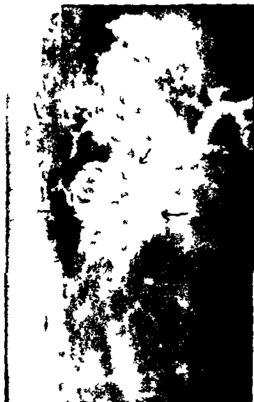


Fig 3 Case 8 Pheo h omocytoma located o the h lus of the left k dney

One yea pre o ly her B P a 225 170 blood non p o e n n rogen 64 mg% eye nd grad 2 heart of normal s ze She ad ed h u p cted ca d a nfar She d cribed her a ta ks as oppr on heart e on and rushing of the blood d he h ad D ng one su h at a k P I f 10 1 as reg ered Ur ne cate hola n re fo nd o be 300 g nor a lrenal n and 31(g ad enal n per 24 hr Glu ose oleran e es ho e l a d abe curv The ba al me bol rate as +23 +29 % E egrounds grade 3 (haemorrhages and exuda es Th h art ze as large (590 r l n bod rfa e R roperi oneal nsufflat on of a r sho ed an o ange sized t mour at the top of the r ht k dney A operation (Hul en the t m ur a ua l be een the k dney and the suprarenal It was removed and found to be a pheochr mocytoma

Case 7

Woman aged 40 Dur ng the previo s 18 months she had attacks of s eat ng mostly of the trunk and the head less pronounced on arms and legs The attacks were not prec p tated by meals emotions or posture A 24 hr ur ne conta ned 1 310—1 100 μg noradrenal ne The basal metabol c rate was +51 +47 % A glucose tolerance curve was abnorn al The B P was 170 100 Operat on (Hulten) fa led to reveal a tumour in the left suprarenal The right suprarenal as accord ngly approached from beh nd and was found to conta n a well capsulated tumour the s ze of a p geon s egg of charac ter s c bro nish appearance M croscopy sho ed a pheochromocytoma

Case 8

Woman aged 33 In No ember 1959 the patient had epistaxis w th a B P of 220/140 a d prote nur a (Esbach 2 ‰) Subsequently she had attacks of tachycard a s eat ng and cool fingert ps pa ns n her forehead (blood ru h ng up) and subst r nal d scomfort irrad ating back ards In 1960 she as adm tted to our Un ers ty Hosp tal th a blood pressure of 240/130 mm Hg ECG affected heart s ze 380 ml/m body surface 24 hr r nes conta ned 325—516 μg oradrenal n and 6 8—28 1 μg adrenal ne Re roper ton al a r insufflat on fa led to re eal an abnormal expan e les on Ho e er aortography ho ed a walnut s zed tu mour in the h lus of the left k dney (f g 3) Th s as surg cally remo ed (Hul én an l f und to be a partially necro c pheochromo- ytoma After recovery her B P was 130 85

Ca e 9

Man aged 77 In 1960 he had a m yocard al farct on In 1964 arterial hypertension 40 120 as noted th ep odes of ery lo arter al pres ure He had paroxysmal achycard a and precord al pa n In 1965 increased catecholam ne excret on was no- d w e X ray n est gat on h re ro- p r oneal ns fflat on of oxyg n sho ed a t mo r at he upper pole of the left k dney

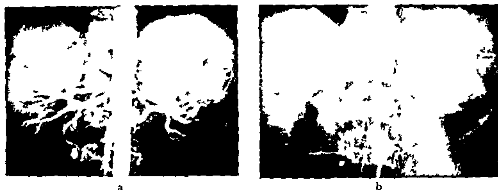


Fig 4 a—b Case 10 Bilateral suprarenal tumour demonstrated at angiography

At laparotomy a tumour in the left suprarenal gland was found and the gland was removed (32 g) Microscopy showed a pheochromocytoma Nor-epinephrine had to be given the first three postoperative days There were signs of kidney insufficiency and heart decompensation The patient improved again after therapy but died suddenly 12 days after the operation

At autopsy old myocardial infarctions and atherosclerosis were demonstrated with nephrosclerosis

Case 10

Boy aged 16 One brother had diabetes

For four or five years the patient had attacks of intense headache in the back of the head at first with an interval of several weeks eventually more often For seven months he had attacks of tachycardia perspiration and dyspnoea For one month he had noticed reduced acuity of vision which caused him to seek medical advice

He was sent to the local hospital where the B P was found to be 260/170 (left arm) and 285/170 (left leg) The fundi presented congested discs Angiography as well as retroperitoneal insufflation of air revealed bilateral suprarenal tumours (fig 4 a—b) Catecholamines in the urine were found to be 650 (84—646) μg noradrenaline and 255 (70—255) μg adrenaline per 24 hours Hydroxy methoxy mandelic acid (HMMA) in urine was 19—28 mg/24 hrs

Preoperative treatment

The total blood volume (TBV) was low (2 67 l = 49 ml/kg body weight) During the week before operation the α blocking drug (phentolamine Regitin®) was given and a β blocker (proprenolol Inderal®) was added Repeated blood tranfusions increased the TBV to normal (4 20 l = 78 ml/kg body weight) preoperatively TBV two days postoperatively was 4.51 l This preparation of the patient was essential to avoid blood pressure fluctuations when the tumour was removed

At operation a tumour the size of a walnut was found in the right suprarenal and one the size of a mandarine in the left The left suprarenal gland was removed (weight 60 g) whereas 20 g were resected from the right leaving a small slice of normal tissue with adequate vascular supply The cut surface was typical of a pheochromocytoma and is shown in fig 5 a and the histological appearance in fig 5 b After the operation the blood pressure settled to 130—100 60—70

No vasopressor drug was necessary to stabilize the B P Hydrocortisone and cortisone were given for ten days HMMA in the 24 hr urine was 1 8 and 2 1 mg noradrenaline 12 6 and 22 5 and adrenaline 2 7—6 0 μg Two months later his condition was entirely satisfactory the fundi rapidly returning to normal and his acuity of vision considerably improved

Comments

The diagnosis of pheochromocytoma may be established in three ways

I At health examination of an apparently symptom free person, in whom the blood pressure is found to be high Case 2 belongs to this type

II At the necropsy of a person who has died from cerebral haemorrhage, pulmonary oedema or cardiac infarction Cases 1 and 3 were of this type

III During life in a person who applies for medical assistance because of certain symptoms and in whom definite signs are observed This group represents the majority of our material (7 cases) The following features should be observed

1 The paroxysmal nature of the attacks is characteristic if encountered On the other hand it should be remembered that paroxysmal hypertension may also be caused by other disorders such as those with severe pain toxaemia of pregnancy or in persons under influence of MAO inhibitors who eat cheese and some other food stuffs Hypertension in pheochromocytoma may be permanent although augmentations during the attacks are still possible Many patients and physicians may ascribe the paroxysm to an attack of migraine or to paroxysmal tachycardia or the like and neglect to register the blood pressure If there is a paroxysmal hypertension it is the rule that the patient is pale and sweating during the attack and after the attack the face may assume a more livid almost plum like colour Precipitating factors are commonly noted, such as pressure exerted by the physician's palpating hand, straining at stool, sexual intercourse, eating a large meal or assuming certain postures involving a pressure on the tumour Hyperventilation may also be a precipitating cause and it should never be forgotten that the diaphragm has an arterial supply in common with the suprarenal In case 1 it is possible that movements of the left arm when lifting heavy objects may have involved a Valsalva procedure eliciting a mechanical effect on the tumour In those rare instances where the pheochromocytoma is located in the wall of the urinary bladder voiding will readily precipitate the attack

Peculiar features may sometimes be connected with the attacks, as in case 4, where the circumference of the neck increased from 39 to 57 cm The same patient reported peculiar pains in his feet, elicited by lifting a heavy object (he was working in an iron foundry) Sharp pains started in the instep and radiated like a flash of lightning" towards the heels This is another illustration of the well known fact that the customer is always right', a matter too easily neglected in a laboratory minded time As emphasized in the literature occurrence in families is by no means unusual in the present small series, case 4 had a brother who died aged 25 from cardiac infarction, which at least is remarkable

2 The blood pressure may range over the whole scale from the level of a moderate benign hypertension to an unmistakeable malignant type, with fundi of grade 4 as in case 10 and case 5 There is however, one feature which may be

a

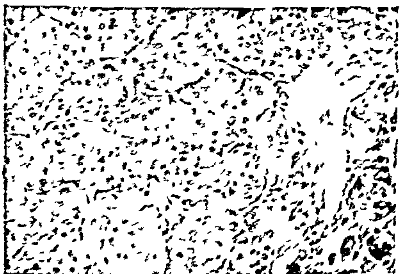


Fig 5 [illegible]

extremely characteristic the pronounced postural reduction of the pressure This feature is no doubt due to the reduced blood volume in these instances a phenomenon contrasting vividly with increased blood volume and absence of postural reactions in primary hyperaldosteronism The reduced blood volume must be considered in the preparation of these patients for surgery (vide case 10) Another feature which may be present is Recklinghausen s neurofibromatosis of the skin or even other neuroectodermosis The localization of the tumour is generally more common in the right suprarenal but it has been considered that neurofibromatosis would favour a left sided localization Our material is too limited to allow any judgement of this point

3 Among laboratory tests we have found the level of the catecholamines in the 24 hour urine by far the simplest, most reliable and least dangerous test and great credit should be given to Ulf von Euler who introduced this method There are sources of error the excretion may be paroxysmal and accordingly not always increased Normal results are obtained if the tumour is located in the urinary bladder False positive results may be obtained if the patient has been given methyl dopa a drug often administered to hypertensive patients by the general practitioner Before the test it is necessary to stop methyl-dopa for at least three days Other tests, praised by many, involve the injection of histamine, which causes an abrupt rise of the blood pressure, or phentolamine (Regitin) which causes a transitory fall of an increased blood pressure but also a rebound phenomenon with an increased pressure afterwards These tests may be reliable but we prefer not to use them, since we know the appearance of the striothalamic arteries in arterial hypertension and the implied danger of bursting of an aneurysm The administration of morphine or its substitutes may also increase the blood pressure in these patients Other laboratory tests may confuse the diagnosis The basal metabolic rate may be increased, suggesting thyrotoxicosis the glucose tolerance curve may be abnormal, suggesting diabetes, and the presence of protein and blood cells in the urine may suggest a renal origin for the hypertension etc

4 Besides the history, the orthostatic nature of the hypertension and the determination of urine catecholamines, by far the most important diagnostic procedure lies with the radiologists Abdominal aortography is to be preferred to retroperitoneal insufflation of oxygen in these cases for two reasons Firstly, these tumours are amply vascularized (in contrast to the Conn tumour) and secondly, the localization of the tumour may in some 10 % of all cases be found outside the suprarenal medulla Tumours can be found at the ventral side of the aorta as in the organ of Zuckerkandl close to the kidney (as in our cases 5 and 8) in the bifurcation of the aorta or in the wall of urinary bladder One of us has suggested this method of examining the suprarenals in 1949 (Ask Upmark E Tillampad anatomi Ahlen & Åkerlund Stockholm published in 1951)

Summary

1 Pheochromocytomas are rare and difficult to identify During the last 11 years in our university hospital we have found 11 pheocromocytomas in ten patients i e about one case a year There are reasons to believe this figure to be an underestimate of the true frequency

2 *Attention is called to the clinical* laboratory and radiological diagnosis of these tumours on the basis of our small series as well as from the observations in the literature The paroxysmal character precipitating factors, marked postural alteration of the blood pressure, catecholamines in the urine and abdominal aortography are our main points

3 'Migraine', 'paroxysmal tachycardia', essential hypertension', "thyrotoxicosis', diabetes mellitus' and 'organic nephropathy' are some of several diagnostic mistakes in these cases

4 The preoperative treatment with blockers (α and in some cases also β) and blood volume expansion to normal values is essential for an uneventful pre and postoperative course

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From the Institute of Hygiene (Head Haakon Natvig M D) University of Oslo, Oslo Norway

Studies on Hemoglobin Values in Norway

IX Hemoglobin, hematocrit and MCHC values in old men and women

By

Odd D Vellar

A number of surveys have been conducted in different population groups in an attempt to learn about the distribution of hemoglobin (Hb), hematocrit and MCHC values in the Norwegian population (6, 7, 8, 9, 10 11, 12 13) In this paper the results of a survey in men and women over the age of 65 years are reported

Material and methods

The present material was obtained at 17 residential homes for old people in Oslo, and at one institution where the men and women lived in selfcontained flatlets The age and sex distribution of the total material are shown in table I

The prevalence of particular well defined diseases was established on the basis of the individual s personal information supplemented by the matron s knowledge of the disease records of the pensioners

All the blood samples were taken from the finger tip by pricking with lancets and Hb and hematocrit determinations were performed by the method described in an earlier study (6) The same well trained nurse took all the blood samples and performed the readings The intake of supplementary iron was also recorded The investigation was carried out from January 1965 to March 1966

Submitted for publication May 18 1967

Results

1 *The total material*

The mean values for Hb hematocrit and MCHC with S D in the different age groups for men and women in the total material are presented in table II

Table I Sex and age distribution of the total material of old people examined

Age (yrs)	Men	Women	Total
65—69	14	45	59
70—74	48	103	151
75—79	76	185	261
80 and over	199	589	788
Total	337	922	1 259

TABLE II Mean hemoglobin hematocrit and MCHC values with S D in the different age groups

Age (yrs)	Men						Women	
	Hemoglobin		Hematocrit		MCHC		Hemoglobin	
	(g %)	(S D)	(%)	(S D)	(%)	(S D)	(g %)	(S D)
65—69	14 71	1 25	44 79	3 41	32 79	1 97	13 97	1 46
70—74	14 31	1 84	43 42	5 42	32 85	2.53	13 75	1 04
75—79	14 67	1 42	44 71	4 40	32 70	1 96	13 34	1 29
80 and over	13 86	1 50	42 32	4 86	32 64	1 85	13 26	1 34

In men the Hb concentration stays fairly constant until 80 years of age, but thereafter decreases markedly In women the Hb concentration gradually falls from 65 years onwards

The mean hematocrit values correspond rather well with the mean Hb concentrations in the respective age and sex groups with the result that the mean MCHC values in both men and women differ only moderately from age group to age group There is no obvious sex difference except for the oldest age group, where women have a significantly lower MCHC value than men of the same age (t 2 4 $p<0\,05$)

2 Men and women with no history of disease

Two hundred and forty men and 695 women had no history of disease The mean values of Hb, hematocrit and MCHC in the different age groups for these individuals are shown in table III In men there is a gradual fall in Hb concentration from 65 years onwards, with a substantial decrease in the oldest age group In women the Hb concentration stays fairly constant from 65 to 74 years, but falls appreciably later in life In men there is also a gradual fall in hematocrit from 65 to 79 years, with a considerable decrease in the age group

TABLE III Mean hemoglobin hematocrit and MCHC in the different age groups for men and women with no history of disease

Age yrs	Men			Women		
	Hemoglobin g	Hematocrit	MCHC (%	Hemoglobin (g %)	Hematocrit (%)	MCHC (%)
65—69	14 78	44 90	32 80	13 74	40 87	32 90
70—74	14 75	44 76	32 90	13 80	41 59	32 91
75—79	14 66	44 32	32 89	13 40	40 85	32 57
80 and over	13 93	42 49	32 65	13 29	40 52	32 30

for men and women in the total material

Hematocrit		MCHC	
(%)	(S D)	(%)	(S D)
42 14	3 28	32 69	1 90
41 53	3 52	32 89	1 49
40 79	3 79	32 54	1 65
40 61	4 14	32 29	1 51

80 and over In women the fall in the hematocrit is not so conspicuous

The mean MCHC values show a moderate fall in men after the age of 80 and a more pronounced fall in women after the age of 75 years After the age of 75 years there is a sex difference of the same magnitude and direction as observed in the oldest age group of the total material

In fig 1 the mean Hb values by age in the normal working population described in an earlier study (13) are compared with the values for the pensioners with no history of disease in the present study In both men and women sharp discontinuities are revealed in the graphs In the age group 65—69 years the gaps represent 0 8 g % Hb in men and 0 7 g % Hb in women in favour of the working population

The corresponding values for hematocrit and MCHC are presented in figs 2 and 3 The breaks in the curves for these parameters also are appreciable In the age group 65—69 years the difference in hematocrit is 1 9 % and in MCHC 0 7 % in both men and women in the same direction as for Hb

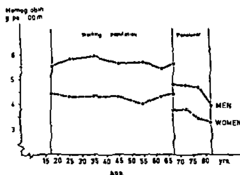


Fig 1 Mean hemoglobin values by age in men and women of the normal working population and of pensioners with no history of disease

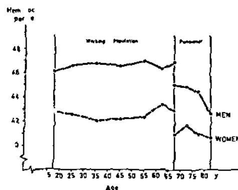


Fig 2 Mean hematocrit values by age in men and women of the normal working population and of pensioners with no history of disease

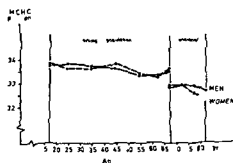


Fig 3 Mean MCHC values by age in men and women of the normal working population and of pensioners with no history of disease

TABLE IV Mean hemoglobin hematocrit and MCHC values among men and women in the control group before and after one month of iron supplement

Time of examination	22 men 88 tablets on average			59 women 90 tablets on average		
	Hemoglobin (g %)	Hematocrit (%)	MCHC (%)	Hemoglobin (g %)	Hematocrit (%)	MCHC (%)
Before iron	13 5	41 2	32 8	13 4	41 0	32 8
One month after iron	13 7	42 0	32 7	13 8	41 7	33 1

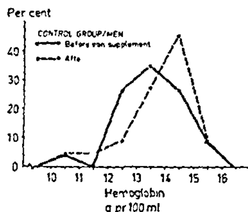


Fig 4 The relative distributions of hemoglobin concentration among men in the control group before and after approximately one month of iron supplement

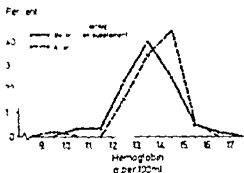


Fig 5 The relative distributions of hemoglobin concentration among women in the control group before and after approximately one month of iron supplement

3 Supplementary iron given to a sample of the total material—the control trial A systematic sample of one in ten of the total material was given iron tablets in order to evaluate the influence of iron supplement on the blood values of old people Individuals however with subnormal MCHC values (less than 30 5 %) and those who already received iron therapy were excluded from this control trial The supplementary iron was given in the form of ferrofumarate (Neo-Fer Nyco supplied by Nyegaard & Co, Oslo) One tablet was equivalent to approximately 60 mg of bivalent iron and the persons received one tablet three times a day Blood controls were taken about one month later and the actual consumption of tablets during the period was recorded All the individuals in question except for one man and two women were re-examined after the period with iron supplements Al together 22 men and 59 women completed the control trial

The response to supplementary iron is shown in table IV and in both men and women there is a tendency towards increased values for Hb and hematocrit

TABLE V Mean hemoglobin hematocrit and MCHC values among men and women in the therapy group before and after one month of iron supplement

Time of examination	28 men 111 tablets on average			65 women 104 tablets on average		
	Hemoglobin (g %)	Hematocrit (%)	MCHC (%)	Hemoglobin (g %)	Hematocrit (%)	MCHC (%)
Before iron	13 3	45 0	29 3	12 6	42 9	29 4
One month after iron	14 4	45 2	31 8	13 5	42 6	31 7

The differences in the mean values however, are not statistically significant except for the increase in Hb for women ($t = 2\ 05$ $p < 0\ 05$) The mean values of MCHC are maintained at a fairly constant level

The relative distribution of the Hb concentration in men and women before and after one month of supplementary iron is presented in figs 4 and 5 The curves demonstrate a shift towards higher values but some low values persist

This seems to indicate that in the control group the iron supplement had a beneficial effect on blood values, and that the dietary iron intake had been insufficient prior to the investigation

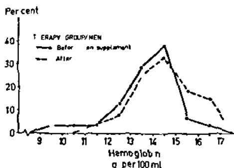


Fig 6 The relative distributions of hemoglobin concentration among men in the therapy group before and after approximately one month of iron supplement (One man with 5 0 g % Hb before iron supplement is not plotted on the graph curve)

4 *Supplementary iron given to persons with MCHC <30.5 %—the therapy trial*

All person with MCHC below 30 5 % who did not receive iron medication were given iron tablets in the same way as in the control group This was in order to assess the influence of iron supplement on the blood levels of indi

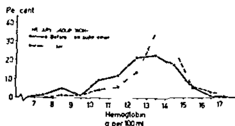


Fig 7 The relative distributions of hemo globin concentration among women in the therapy group before and after approximately one month of iron supplement (One women with 20 2 g % Hb after iron supplement is not plotted on the graph curve)

TABLE VI. The frequency of low hemoglobin concentration and subnormal MCHC values in men and women in different age groups

Age (yrs)	Men: per cent with		Women: per cent with	
	Hemoglobin <14.0 g %	MCHC <30.5 %	Hemoglobin <12.5 g %	MCHC <30.5 %
65–69	28.6	14.3	11.1	6.7
70–74	39.6	6.3	9.7	5.8
75–79	31.6	11.8	21.1	7.6
80 and over	48.6	12.1	22.8	12.7
Total	42.7	11.3	20.4	10.6

viduals with subnormal MCHC values. Altogether 28 men and 65 women completed this trial.

The mean Hb, hematocrit and MCHC values before and after supplementary iron are presented in table V. In both sexes an appreciable and statistically significant increase in Hb concentration is achieved (for men $t = 2.6$, $p = 0.01$, for women $t = 3.0$, $p < 0.01$). The mean values of the hematocrit, however, stay fairly constant, which results in a substantial increase in the mean MCHC values comparable to the increase in the mean Hb concentrations. In five men and 14 women, however, MCHC was still below 30.5 % after the trial.

The relative distributions of Hb concentration before and after iron supplement are shown in figs. 6 and 7. In both sexes there is a shift towards higher values, but the long tail of low values is not eliminated.

The therapy trial clearly demonstrates that the majority of subnormal MCHC values are corrected with supplementary iron, which also gives higher Hb values.

5. The frequency of low hemoglobin concentration and subnormal MCHC values

According to previous findings (13) anemia is considered to exist when the Hb concentration is less than 14.0 g % in adult men, and less than 12.5 g % in adult women under the age of 70. MCHC values below 30.5 % in both sexes are also considered subnormal.

The frequency of low Hb concentration and subnormal MCHC values of the total material according to these criteria is shown in table VI. In men an Hb concentration of below 14.0 g % is very frequently found in all age groups, but is most pronounced after the age of 80 years. In women an Hb concentration below 12.5 g % is prevalent in all age groups, more especially after the age of 75 years.

In both men and women low Hb concentrations are substantially more frequent than subnormal MCHC values. There is a tendency, however, towards an increased proportion of subnormal MCHC values with increasing age.

Discussion

The present survey was not undertaken in a random sample representative of old people in the general population For practical reasons it had to be limited to institutions for the aged, for otherwise the fulfilment of the supplementary iron trials would have been impossible The principal results of this investigation might, however, be relevant to old people in Oslo in general in spite of differences in conditions of living, dietary habits, financial status health conditions etc

In a previous study of the working population (13) it was found that men aged 30—39 years had a maximum Hb level, with subsequently lower values for each ten year age group after this age The present study shows that the decreasing tendency is continued after the age of 65 years and that the difference between the mean values of the Hb concentration for the age groups 30—39 and 80 years and over, is in the total material 1 9 g % and in the normal material 2 0 g %

The same tendency for a gradual decrease in Hb concentration with increasing age is encountered in women but the absolute reduction is not of the same magnitude as revealed in men In all age groups the Hb level for women is appreciably lower than that for men, though the difference is much less marked after the age of 80 years This study has confirmed previous findings by Walsh et al (15), who also found a progressive reduction of Hb values with *increasing* age in both sexes Kilpatrick (4) however, found a fairly steady decrease with age in men, but not in women Orchard (14) reported similar results, and even revealed a rise in the Hb level above the value of men in normal women after the age of 84 years Hawkins et al (3) also found that the Hb values of women in their eighties tended to be higher than those in men

The hematocrit and MCHC values of the old men and women in the present study are also lower than those found in the working population This is in contrast to previous findings by Orchard (14), who found that the two parameters were within normal limits in men and women between the ages of 65 and 99 years Beyond the age of 84 years, however the men showed a decrease, and the women an increase in hematocrit The material, however, was comprised of individuals who were free from diseases known to influence hemopoiesis

In all indices there is a break in the curves for both sexes (figs 1, 2 and 3) in the age group 65—69 years, in favour of the working population (13) Small numbers in the different groups may be responsible for the discrepancies to a great extent, but a real biological difference in hematological status between the working population in their late sixties and retired men and women of the same age cannot be ruled out All subjects were screened in order to exclude definite pathological conditions but the screening was more thoroughly carried out for the working population Previous studies (1 15) have shown that the mean Hb level of blood from the finger does not differ significantly from that of venous blood but there is some

variability in individuals. Thus differences in the site of blood collection in the two studies cannot be responsible for the discontinuities in the curves.

Both men and women with MCHC values below 30.5 % reached a higher mean Hb and MCHC levels after the iron supplement, but some low values still persisted. Thus supplementary iron had a beneficial effect on blood values after approximately one month, and thus indicates that the dietary iron intake was insufficient prior to the investigation. The results of iron supplementation in the control group support this view. There is reason to believe that the response in both groups might have been even more pronounced if the period of treatment had been longer. Dietary surveys in old age and nursing homes (2) have revealed that the intake of iron was substantially below the standards.

Our previous studies have shown that iron deficiency anemia is rather prevalent among school children (12), adolescent grammar school pupils (12), young men (6, 11), women in the reproductive age, and men after the age of 40 years (13). We do not know what should be considered as normal values for Hb and MCHC in old people. The frequency of low Hb concentration, however, according to the criteria for adult men and women under the age of 70 years, was very high in old men and considerable in old women. The frequency of subnormal MCHC values was also pronounced, but not to the same extent as the Hb concentration. Previous studies of younger age groups have disclosed that subnormal MCHC values were encountered more frequently than low Hb concentrations (6, 11, 12, 13). This may indicate that the lower limits for normal Hb concentration should be less than 14.0 g % in old men and less than 12.5 g % in old women. The high frequency of subnormal MCHC values, however, show that sideropenic anemia is rather prevalent in old people. These results are in agreement with previous findings by Lange and Skjeggestad (5) who, among patients hospitalized in the Oslo City Hospitals, found that sideropenic anemia was encountered more frequently after, than before, the age of 50 years.

It must be stressed that anemia is a symptom and not a diagnosis, and the possibility of bleeding must not be forgotten. Some of the old men and women in this study had grave anemia with only vague symptoms or none at all, and it seems necessary to determine Hb routinely in the aged, in order to detect diseases as early as possible. The practical approach to this problem may be solved in a number of ways.

Summary

The Hb, hematocrit and MCHC values have been determined in 337 men and 922 women living in 17 residential homes for the aged and in one institution with self-contained flatlets, all in Oslo (table I).

The mean Hb, hematocrit and MCHC values are presented in table II for the total material, and in table III and figs. 1, 2 and 3 for individuals with no history of disease.

In a control trial performed in a systematic sample of the total material

supplementary iron was given for about one month with subsequent determination of the three blood indices The response shown in table IV and figs 4 and 5 indicates in the control group that iron supplements had a beneficial effect on the blood values in both men and women

In a corresponding therapy trial men and women with MCHC below 30 5 % were given supplementary iron according to the same scheme Table V and figs 6 and 7 demonstrate a marked increase in both Hb and MCHC values

Low Hb concentration and subnormal MCHC values (Hb below 14 0 g % in men and below 12 5 g % in women MCHC values below 30 5 % in both sexes) are frequently encountered in men and women after the age of 65 years (table VI)

The most likely explanation of these findings is that the dietary iron intake of the aged is inadequate

Acknowledgement

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Viral Hepatitis

A follow up study of 373 notified cases in Oslo 1949—1953

By

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Acute viral hepatitis is widespread in many countries There are many prophylactic thereapeutic and prognostic problems related to the disease

Notification of *hepatitis infectiosa* in the weekly or monthly records was introduced in Norway in January 1942 During the first year of notification about 27,000 cases were notified There has since been a big fall in the number of new cases per year though some fluctuations from year to year have occurred In Sweden and Denmark there has been a similar epidemiological situation During the last few years the number of notified cases per 100 000 inhabitants has fallen below 50 per year in all three countries

To help assess the incidence of the disease, the principle of nominative notification was introduced in Norway from January 1966

During recent years there have been lawsuits after outbreaks of epidemics of hepatitis caused by infected oysters from Havstensund in Sweden and by polluted drinking water in a winter sport resort in Norway The prognosis of the patients has been one of the most important questions in the lawsuits

The immediate lethality of caute hepatitis varies Natvig (7) reported a lethality of 0 53 %, based on the Norwegian health statistics from the period 1942—47 Barker et al (1), however found only 0 18 % among U S soldiers in the Mediterranean theatre during World War II Kalk (5) also reckons about 0 2 % but reports outbreaks with appreciably higher figures A number of authors (5, 6 7 11) stress the fact that serum hepatitis (inoculation hepatitis) seems to have a more serious prognosis than infectious hepatitis (epidemic hepatitis)

It has been difficult to ascertain the frequency with which long term sequelae develop after acute viral hepatitis as most of the observations deal with selected cases admitted to hospitals, and there

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have been no adequate controls Thus, Siede and Klamp (10), who reviewed the results of 16 different investigations, found that the incidence of cirrhosis after acute hepatitis varied from 0 1 to 33 % Chuttani et al (3), however, in their five year follow up study of unselected cases from the Dehli epidemic of 1955—56, could not find any definite clinical evidence of persistent liver damage among 304 persons studied In none of the epidemic patients who had died between the epidemic and the follow up study, could the terminal state be attributed to liver disease

There are reports of the development of sub-acute liver atrophy and chronic hepatitis especially after the age of 40 years (2 4) Bjorneboe et al (2) found a high incidence of chronic hepatitis in municipal hospital departments in Copenhagen in 1944—45 Ninety seven % were in women and the case lethality was 37 % The great majority of the patients were women over 40 years of age Wang (14) revealed that hepatitis was the underlying cause of death in at least 10 % of cases with cirrhosis of the liver in a big post mortem series from hospitals in Oslo 1929—45 In 1944—45 the combination of previous hepatitis with death from liver cirrhosis was especially encountered in women in accord with the observation from Copenhagen (2)

The main objective of the present follow-up study was to ascertain the immediate lethality from viral hepatitis and the long term prognosis in unselected cases of hepatitis notified in the general population of Oslo 1949—53

TABLE 1 The material of the follow up study

	Number	Per cent	
Notified cases of hepatitis in Oslo 1949—53	373	100 0	
The follow up study			
Returned questionnaires	266	71 3	87 1
Deceased subjects	59	15 8	
Unreturned questionnaires + insufficient information	48	12 9	

Material and method

In 1954 Natvig and Adelsten Jensen (8) reported the results of an epidemiological study of viral hepatitis notified in Oslo in the three year period 1.XI 1949—31.X.1952 In all notified cases detailed additional information was collected in order to ascertain the diagnosis and to assess the particular epidemiological situation. The material consisted of 316 cases of hepatitis, 134 men and 182 women. The present follow up study was accomplished in the spring of 1966 12—16 years after the acute attack of hepatitis The material studied consisted of 316 cases from the previous study (8) with addition of 57 cases notified in Oslo in the period 1 XI 1952—31 X 1953 Ascertainment of diagnosis and assessment of epidemiological circumstances had also been performed in these cases

Among the 373 cases in the total material 220 were designated as infectious hepatitis 75 as serum hepatitis and 78 as possible serum hepatitis At the time of the follow up study a questionnaire was sent to all survivors who were asked to inform about any recurrence of jaundice the existence of other serious diseases and admittance to hospital The death certificates from the 59 subjects who had died during the intervening period, were collected and studied and additional information from doctors and hospitals was examined in 28 cases where liver and bili

TABLE II Age at the time of the acute attack of hepatitis in the final material

Age (yrs)	Men			Women		
	Returned questionnaires + deceased subjects	Unreturned questionnaires + insufficient information	Total	Returned questionnaires + deceased subjects	Unreturned questionnaires + insufficient information	Total
0—9	4	1	5	5	1	6
10—19	10	0	10	8	2	10
20—29	35	6	41	33	10	43
30—39	30	8	38	49	9	58
40—49	24	3	27	34	2	36
50—59	16	2	18	26	2	28
60—69	8	1	9	20	0	20
70—79	4	0	4	7	0	7
80—89	2	0	2	0	0	0
Unknown	0	0	0	0	1	1
Total	133	21	154	182	27	209

ary tract diseases carcinomatosis etc were recorded as the cause of death i e in all cases where pathological liver function might have been present The material of the follow up study is presented in table I

When the hospital records were studied ten of the deceased subjects had to be excluded from the material because the diagnosis had not been acute hepatitis when notified in 1949—53 In five of these cases the diagnosis should have been chronic hepatitis and in five obstructive jaundice The final material consisted of 363 persons 154 men and 209 women with a total of 49 deaths Table II shows the ages at the time of the acute attack of hepatitis

Results

The cause of death in the 49 deceased subjects is shown in table III The causes have been divided into three groups, based on the degree of relationship between the initial attack of acute hepatitis and the terminal state

In group I the cause of death was directly related to liver failure following the attack of hepatitis and caused by immediate complications or subacute and chronic liver derangements This group consisted of seven persons or 1 9 % of the final material There were six women and only one man, and all the subjects had probably had infectious hepatitis and not serum hepatitis in the initial attack

One man and one woman 45 and 60 years old respectively died during the acute attack of the disease Thus the immediate mortality was 0 6 %

Death from liver failure in the subacute and chronic phase of the disease occurred in five women, or 2 4 % of the women in the final material No death *in men was recorded in this category* The case histories of the five women are given below

TABLE III The cause of death in 49 subjects who had died in the follow up period

	Men	Women	Total
I Hepatitis directly related to liver failure with death			
Acute hepatitis	1	1	2
Subacute liver atrophy	0	1	1
Chronic hepatitis/cirrhosis of the liver	0	4	4
II Hepatitis probably contributing to death			
Cirrhosis of the liver (congenital portal hypertension)	1	0	1
Coronary heart disease (moderate liver cirrhosis at post mortem)	1	0	1
Lipo-fibrosarcoma with metastases (liver cirrhosis as clinical diagnosis)	1	0	1
III Hepatitis unrelated to the cause of death			
Cholelithiasis reoperated	0	1	1
Cancer/carcinomatosis	3	4	7
Heart, vascular pulmonary and renal diseases	10	17	27
Mors subita cause of death unknown and insufficient information	3	1	4
Total	20	29	49

Case 1

Born 1878 Woman notified as hepatitis January 1952 aged 74 years Admitted to hospital in February and died in July the same year seven months after the onset of the disease Post mortem diagnosis subacute yellow atrophy of the liver (Diagnosis from death certificate acute hepatitis)

Case 2

Born 1941 Girl notified as hepatitis November 1950 aged nine years Admitted to hospital a number of times in 1950—52 She died in hospital May 1952 1 1/2 years after the onset of the disease Post mortem diagnosis chronic hepatitis (Diagnosis from death certificate chronic hepatitis)

Case 3

Born 1903 Woman, notified as hepatitis April 1951, aged 48 years Previously admitted to hospital for narcomania pyonephrosis and secondary polyarthritis (She was not taking injections) Admitted to hospital March 1951 for hepatitis and died in November the same year eight months after the onset of the disease Post mortem diagnosis cirrhosis of the liver, left sided paranephritic abscess with fistula. (Diagnosis from death certificate hepatic cirrhosis)

Case 4

Born 1885 Woman, notified as hepatitis September 1950 aged 65 years Hospital treatment for hepatitis a number of times 1950—52 She died November 1952, two years and two months after the onset of the disease Post mortem diagnosis cirrhosis of the liver (Diagnosis from death certificate chronic hepatitis)

Case 5

Born 1887 Woman, notified as hepatitis May 1953 aged 66 years In 1952 myocardial infarction. Admitted to hospital June—August 1953 for acute hepatitis Readmitted and died December the same year seven months after the onset of the disease Post mortem examination not performed. (Diagnosis from death certificate hepatic cirrhosis)

In group II hepatitis may probably have contributed to the death, but not directly related to the disease causing it. Three deaths among men were registered in this group. The case histories are given below

Case 6

Born 1935. Man, notified as hepatitis October 1950 aged 15 years. At the age of nine years however hepatosplenomegaly was diagnosed. Admitted to hospital October 1950 for acute hepatitis splenomegaly and hepatomegaly. Readmitted August—October 1955 for hepatic cirrhosis obliteration of the portal vein. He died in July 1957, nearly seven years after the apparent attack of *acute hepatitis. The condition was interpreted* as a congenital obliteration of the portal vein with subsequent development of portal hypertension. Post mortem diagnosis not available. (Diagnosis from death certificate hepatic cirrhosis.)

Case 7

Born 1882. Man notified as hepatitis February 1950 aged 68 years. December 1949 myocardial infarction. Admitted to hospital February—June 1950 for hepatitis. Treatment in hospital a number of times 1954—57 for sideropenic anaemia arteriosclerotic heart disease. Readmitted June 1959, and died September the same year nine years after the attack of hepatitis. Post mortem diagnosis coronary sclerosis hypertrophy of the heart pulmonary oedema and moderate cirrhosis of the liver. (*Diagnosis from the* death certificate hepatic cirrhosis.)

Case 8

Born 1908. Man notified as hepatitis February 1950 aged 42 years. Admitted to hospital February—March 1950 for acute hepatitis and in 1962 for cirrhosis of the liver and peptic ulcer of the stomach. Readmitted April 1963 and died June the same year 13 years after the attack of acute hepatitis. Clinical diagnosis chronic alcoholism cirrhosis of the liver (primary liver carcinoma). Post mortem diagnosis lipofibrosarcoma with metastases to peritoneum and liver bronchopneumonia. (Diagnosis from death certificate hepatic cirrhosis.)

TABLE IV The occurrence of liver and biliary tract diseases in the observation period according to the returned questionnaires

Diagnosis	Men	Women	Total
Recurrence of hepatitis	1	1	2
Chronic hepatitis	*0*	*2*	2
Biliary tract diseases	7	5	12
Pregnancy complicated with jaundice	—	1	1
No liver or biliary tract diseases	105	144	249
Returned questionnaires total	113	153	266

Thirty nine cases were allocated to group III where hepatitis appeared to be unrelated to the cause of death

Among the 266 subjects who completed the questionnaire, presented in table IV two persons or 0.6 % of the material had an episode of liver illness assumed to be a recurrence of hepatitis. A 21 year old man was hospitalized for liver disease in the year following the initial attack of hepatitis but later on he *had no symptoms of liver disease*. A woman aged 55 years at the time of the acute hepatitis had a recurrence of jaundice 12 years later

The diagnosis chronic hepatitis had been used to describe the condition in two young women. A 19 year old woman later married gave birth to two babies five and eight years after the onset of hepatitis. The pregnancies seemed to have a beneficial effect on her liver

disease, suggestive of the subsidence of the disease process rather than of the emergence of an underlying chronic condition A 32 year old woman was hospitalized in 1959 and 1960 for chronic hepatitis with 'spider' nevi, six and seven years after the acute attack of hepatitis respectively She is still under observation for chronic liver disease 13 years after the initial attack, and cortisone treatment is administered at intervals

Twelve persons had in the observation period been treated for biliary tract diseases, and one woman had jaundice as a complication during pregnancy, as shown in table IV

Discussion

In this follow up study which was conducted 12—16 years after the acute attack of hepatitis, it was possible to trace nearly 90 % of the original material Earlier follow up studies with such a long observation period are not published, as far as is known Our material consisted of all notified cases of hepatitis in Oslo in a four year period, i e an unselected material This is in contrast to the investigation by Barker et al (1) whose report was restricted to military personnel, or the observation by Bjørneboe et al (2) who dealt with selected cases admitted to hospitals Only the 5 year follow up study of cases from the Dehli epidemic of infectious hepatitis of 1955—56 by Chuttani et al (3) is comparable to our investigation as their material belonged to the general population of Dehli of both sexes

In our material, the immediate lethality from the acute attack was 0 6 % which agrees with Natvig (7) who reported 0 53 % from the official health statistics of the general population in Norway 1942—47

Five women, or 2 4 % of the women in the material, died in the subacute or chronic phase of the disease and most of them had passed the menopause These findings are in agreement with earlier observations (2, 4) Based on the results from the questionnaires, it is probable that another two women suffered from chronic liver derangement but were still alive at the time of the follow up study

Three men died with a clinical or post mortem diagnosis of liver cirrhosis All had another serious disease, however, not related to the liver, as the direct cause of death, or, in addition to the previous attack of hepatitis, another liver disease which might explain the development of cirrhosis There is reason to believe that in none of them could the terminal state be directly attributed to the previous attack of hepatitis, but it might have been a contributory factor These deaths occurred more than six years after the acute attack of hepatitis, in contrast to the corresponding deaths in women who all died less than three years after the attack, and in whom the cause of death was supposed to be directly related to the hepatitis

The probable rate of recurrence of hepatitis in our material was only 0 6 %, compared with 7 8 % as reported by Neefe et al (9) in their 4—6 years follow up study They found that the rate of recurrence was a markedly decreasing function of time and concluded that the occurrence of an episode of liver

disease more than three symptom free years following an initial attack of hepatitis should make one consider the probability of a new infection or other process, rather than an exacerbation of the original disease

Other studies (5, 6, 7, 11) have documented that the prognosis in serum hepatitis is more serious than in infectious hepatitis The seven subjects in our material, however, who died from the acute attack or during the subacute or chronic phase of the disease, probably all had infectious hepatitis

It must be concluded from the results of this study and earlier observations that serum hepatitis as well as infectious hepatitis is a disease which must be treated seriously The immediately lethality in our material is not negligible, and this is in a country with a high standard of living good nutrition and well developed health services Deaths in the sub-acute and chronic phase were rather prevalent, and therefore the long term prognosis must be judged with care in each individual case This is especially relevant to women who have passed the menopause Our observations seem to indicate that the first three years after the acute attack are most important in this respect

The official death certificates are important records also in follow up studies It must be stressed however that the information in these certificates must be scrutinized with caution, and supplemented with data from doctors and hospitals when possible The diagnosis on the certificate may be incomplete or directly misleading as in some of our cases

It is a sincere hope that introduction of nominative notification of hepatitis will contribute to a more complete picture of the epidemiology of this disease in Norway, thus rendering opportunities for a better control of the disease It is important that patients with hepatitis be treated as reservoirs of infection They should not be placed in open wards and the risk of parenteral transmission of virus must not be forgotten The use of disposible equipment for injections etc is important Medical personnel at all levels must be instructed in the preventive measures

Hepatitis in track finders is a new problem, but must be taken seriously A protective competition dress and better hygiene in connection with washing after competitions are the most important prophylactic measures (12, 13)

Summary

Three hundred and seventy three unselected persons who were notified as hepatitis in Oslo 1949—53 were assessed in this follow up study 12—16 years after the acute attack of hepatitis 87 1 % returned the questionnaires or were dead at the time of the investigation (table I) The age and six distribution of the material is presented in table II

The immediate lethality from the acute attack was 0 6 %

Five women or 2 4 % died from subacute yellow atrophy of the liver, chronic hepatitis or cirrhosis during the first three years after the onset of the disease (table III) Most had passed the menopause

Another two women had persistent

liver derangement (chronic hepatitis), but were still alive at the time of the investigation (table IV)

There was a history of probable recurrence of hepatitis during the observation period in two subjects or 0 6 % (table IV)

The long term prognosis of infectious hepatitis seems to be rather favourable in men and young women, but serious in women who have passed the menopause

In all cases in this study where the death was directly attributed to the hepatitis, the initial attack had probably been *infectious hepatitis* and *not serum hepatitis*

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Serum Lipids in Male Patients Hospitalized for Peptic Ulcer

By

RICHARD HELLSTROM

Several authors (2 20, 25) have found in post mortem studies that peptic ulcer patients run a significantly greater risk of death from myocardial infarction if they live on a high milk fat diet as earlier recommended by Sippy in 1915 (23) That the composition of the diet affects the cholesterol and triglyceride concentration in serum has been pointed out by other authors (1, 8, 19) Earlier investigations (4, 14) have also shown that excess weight leads to elevation of the serum lipids No previous investigation has been made as to whether the patients hospitalized during 1965 for peptic ulcer today maintain a gastric ulcer diet or otherwise live under conditions such as to produce a high cholesterol or triglyceride level in serum The same applies to the frequency of myocardial infarctions in such patients These conditions have therefore been specially considered in this study

Previously all peptic ulcer patients were kept in bed at hospital for 3—4 weeks whether haemorrhage was present or not (12, 18 21) The composition of the diet was at that time thought to be of very great significance for the cure (12, 23) The opinion on these questions has since changed (7 13 22) Especially in order to throw light on these points the present study reports the length of time the patients were kept in hospital, the frequency of complicating haemorrhages and the frequency of surgery in direct association with hospital treatment

Material

The material consists of 76 men who were admitted to the Medical Clinic of the Danderyd Hospital on a diagnosis of ventricular or duodenal ulcer during 1965 The diagnosis was in all cases confirmed radiologically either as a distinct niche or as a distinct deformation of the bulb In the original material six patients were found to have diabetes mellitus These cases have been excluded Since discharge from hospital five patients had died before the follow up could be made Two patients furthermore were lost trace of Since the missing patients included six cases of diabetes and anyway do not constitute a selected material they

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cannot have affected the final results to any appreciable extent Of the original 76 cases there thus remain 63 (83 %), all of whom were followed up It should be specially pointed out that all patients derive from the hospital s reception area in Stock holm County and not from the remainder of the country

Methods

In the follow up examination measurements were made of height weight and blood pressure (diastolic pressure at the moment when the Korotkow sounds rapidly diminished in loudness) The patients ages were also recorded Measurements were made too, of the skinfold thickness on the medioclavicular line at the level of the navel and of the femoral condyle width in the right knee with the knee at an angle of 90° by the method reported earlier (15 16 17)

The blood samples were collected in the morning on a fasting stomach Determinations were made of cholesterol and triglyceride levels in serum by the earlier described method (3 5) and also of the sedimentation rate white count haemoglobin concentration, serum iron and transferrin The urine was analysed for protein and sugar

Every patient was personally questioned by the examiner as to whether he kept any form of gastric ulcer diet or not, whether he avoided milk butter and fatty food and whether he took nicotine acid drugs or not The patient was also asked whether he had *previously had a gastric ulcer or myocardial* infarction He was asked, too whether he considered he was suffering particularly from mental stress at the time of onset either because of his work or for other reason

Notes were made of the period in hospital whether the ulcer was duodenal or ventricular whether or not haemorrhage occurred, whether the patient was immediately transferred to the surgical clinic or not Finally all patients were asked whether either parent had had gastric ulcer or myocardial infarction

Results

The distribution of the peptic ulcer patients by duodenal and ventricular ulcer is shown in table I As will be seen, 46 (73 0 %) had duodenal and 16 (25 4 %) ventricular ulcer One patient had a duodenal and ventricular ulcer simultaneously Only as regards body weight and sedimentation rate was there a probably significant difference between the groups

No less than 52 patients (82 2 %) had haemorrhage on admission to hospital (table II) with lowering of Hb concentration to an average of 8 7 g/100 ml Of the 46 patients with duodenal ulcer 41 (89 1 %) had haemorrhage, in the form of melaena alone in 54 % and of haematemesis with or without melaena in 46 % Of the 16 ventricular ulcer cases ten (62 5 %) had haemorrhage, 20 % in the form of melaena alone, 80 % haematemesis with or without melaena

The mean time of hospitalization for the entire material was 14 1 days There were no significant differences in this respect between the duodenal and ventricular ulcer cases, nor between the haemorrhagic and non haemorrhagic Nor was the mean hospitalization time *affected by the fact that some patients* bled so much that blood transfusions were considered necessary

From table II it will be seen that eight patients (12 6 %) were operated on Most of these were duodenal ulcer cases Three of the cases (4 7 %) had such serious haemorrhage after admission that an acute phase operation had to be performed within the first 2—3 days Of the remaining five cases four

TABLE I Differences between patients with ventricular and duodenal ulcer

	Ventr ulcer		Duod ulcer			
	n	$\bar{x}$	n	$\bar{x}$	Diff	P
Age years	16	51 8	46	48 1	3 7	>0 2
Height cm	16	172 3	46	175 8	3 5	>0 1
Femoral condyle width cm	16	9 90	46	10 11	0 21	>0 1
Body weight kg	16	68 6	46	75 6	7 0	x
Skinfold thickness mm	16	15 1	46	18 5	3 4	>0 1
Systolic B P mm Hg	16	137	46	140	3	>0 2
Diastolic B P mm Hg	16	88	46	90	2	>0 2
Cholesterol mg/100 ml	16	245	46	249	4	>0 2
Triglycerides mg/100 ml	16	104	46	94	10	>0 2
ESR mm/hr	16	13 4	46	6 8	6 6	x
White blood cells/mm³	16	6 006	46	5 667	339	>0 2
Hb concentration g/100 ml	16	14 5	46	14 8	0 3	>0 2
Serum iron gamma %	16	127	46	127	0	>0 2
Transferrin gamma %	16	346	46	354	8	>0 2

TABLE II Duodenal and ventricular ulcer patients with and without haemorrhage

Peptic ulcer patients	n	Age	Mean time in hospital	Lowest Hb conc	Operation	
					Acute phase	Non acute phase
Duodenal ulcer	46	48 1	14 2	9 3		
With haemorrhage	41	47 7	14 [illegible]	8 7		
Transfusion	18	49 3	14 6	7 2	2	3
No transfusion	23	46 4	14 5	9 8		2
Without haemorrhage	5	51 0	11 4	14 2		
Ventricular ulcer	16	51 8	14 7	7 9		
With haemorrhage	10	53 4	1[illegible] 3	[illegible] 8		
Transfusion	3	[illegible]4 4	16 7	6 4		
No transfusion	7	[illegible]3 0	14 7	[illegible] 8		
Without haemorrhage	6	49 1	13 7	14 1		
Duodenal + ventricular ulcer	63	49 0	14 1	9 6		
With haemorrhage	52	4[illegible]	14 4	[illegible] 7		
Transfusion	22	4[illegible]	14 [illegible]	7 2	3	3
No transfusion	[illegible]	4[illegible] 0	14 [illegible]	[illegible] 8		2
Without haemorrhage	11	[illegible]0 0	12 [illegible]	14 2		

¹ This group includes one patient with both duodenal and ventricular ulcer

TABLE III Values recorded at follow up one year after hospitalization for gastric ulcer

	n	$\bar{x}$	SD	ε_x
Age years	63	49.0	13.3	1.7
Height, cm	63	175.1	7.7	1.0
Femoral condyle width cm	63	10.1	0.5	0.1
Body weight kg	63	74.0	12.0	1.5
Skinfold thickness, mm	63	17.6	8.1	1.0
Systolic B P mm Hg	63	139.1	23.1	2.9
Diastolic B P mm Hg	63	89.2	12.1	1.5
Cholesterol mg/100 ml	63	249.0	50	7•
Triglycerides, mg/100 ml	63	98.6	53.5	6.7
ESR mm/hr	63	8.8	10.2	1.3
White blood cells/mm³	63	5 757	2,248	283
Hb concentration g/100 ml	63	14.7	1.2	0.1
Serum iron gamma %	63	126	43	5
Transferrin gamma %	63	354	60	8

were operated on for recrudescent minor haemorrhage which did not stop after an average of three weeks treatment. In the fifth case an operation was done after five days of treatment for a recrudescent ulcer which had not previously bled. All patients came through the operations well.

The mean age body dimensions and blood sample results will be seen from table III. The mean age, 49.0 years (range 21—83) does not differ markedly from that in Tomenius study (24). In the present study 17.4 % of the patients were over 60 years of age. The body weight (table III) must be considered to be largely normal in relation to height. The mean skinfold thickness was, however greater than for conscripts of corresponding skeletal dimensions (9).

As regards the mean cholesterol (249 mg/100 ml, range 156—369) and triglyceride levels (98 mg/100 ml, range 26—262) in table III, these did not exceed the figures earlier reported as normal for the age of these patients (3). Eight of the patients had cholesterol levels above 300 mg/100 ml and in nine the triglyceride levels were above 150 mg/100 ml. No significant correlation was found between, on the one hand, cholesterol and triglycerides and, on the other, age, weight, height, femoral condyle width and skinfold thickness. Furthermore, only nine patients (14.1 %) stated that they kept a mild form of ulcer diet, and three that they particularly avoided fat. These 12 patients did not noticeable differ from the remainder of the material as regards blood fats and skinfold thickness.

Forty one (65.0 %) of the 63 patients considered that their mental stress had been such as to have an active part in bringing on the illness. It also appeared that an equal number of patients (65.0 %) had previously had gastric

ulcer, 19 between the years 1961—1965, 16 between 1956—1960, and six prior to 1956 Finally it appeared that no patient in the material had earlier had myocardial infarction

Eighteen of the patients (28 5 %) had had a parent with gastric ulcer, and four (6 3 %) a parent with myocardial infarction One parent had had both myocardial infarction and peptic ulcer

An analysis has also been made of the causes of death of the five patients who died before a follow up examination could be made In two cases the diagnosis was dementia senilis in one cancer vesicae urinariae, in one cancer of the lung and in one cancer of the stomach The latter had been operated on for ventricular ulcer in 1966

Discussion

Sixty three patients hospitalized in 1965 for peptic ulcer have been followed up one year later Of these patients 73 0 % had duodenal and 25 4 % ventricular ulcer This distribution agrees closely with earlier studies (12, 18 21, 24)

Haemorrhage occurred in 82 2 % of the patients, a very much higher figure than in earlier studies (10 11, 12, 18) A change thus appears to have occurred Gastric ulcer patients are now generally hospitalized only if haemorrhage is present Thus is the rule at least at Danderyd Hospital, and probably at most other hospitals too

The average time of hospitalization was 14 1 days This is considerably shorter than was usual earlier (12, 18) Thus, although the number of haemorrhage cases has increased relatively the time in hospital has diminished, which must be considered to be due chiefly to a changed attitude to hospital care and treatment The hospitalization period was not longer in the case of patients who initially had such severe haemorrhage that blood transfusion must be given. This is in close accordance with the results of a recent report (11)

No significant difference was found between duodenal and ventricular ulcer patients (table I) This does not agree with Wretmark s findings (26), that duodenal ulcer patients were taller and thinner than those with ventricular ulcer This discrepancy from Wretmark s results as regards body build is undoubtedly due to the different composition of the present material in which 82 2 % of the patients had gastric haemorrhage

Of the peptic ulcer patients 65 0 % considered they had been under such mental stress prior to the onset that this had an active part in bringing on the illness Similar figures have been reported earlier (11 26) The percentage of patients who had had a parent with gastric ulcer was 28 5 % and 65 0 % had themselves had a gastric ulcer on some previous occasion Both these percentages are relatively high compared with earlier reports (6, 18) Heredity and earlier occurrence of gastric ulcer would thus also appear to be important factors when assessing the risk of this ailment Nevertheless the gastric haemorrhage and ulcer symptoms dissappeared as a rule very quickly after the patients had become settled in mind after admission to hospital This may indicate that mental stress which was reported in 65 0 % of the cases was the chief factor in bringing on the attack

The mean level of cholesterol and triglycerides in serum was normal (table III) compared with earlier studies (3) The ulcer patients in the present study, therefore, would not seem, on an average, to have had a blood fat raising diet None of the patients moreover, had had myocardial infarction, although 17 4 % of them were above 60 years of age Thus no relation has been found between peptic ulcer and myocardial infarction, which is contradictory to earlier results (2, 20, 25)

Summary

All patients treated at Danderyd Hospital during 1965 for peptic ulcer were followed up one year later Of the original 76 cases six patients with diabetes mellitus were excluded Five of the patients had died before the follow up examination could be made Two other patients had been lost trace of All the remaining 63 cases (83 0 %) were examined

Forty six patients had duodenal (73 0 %) and 16 (25 4 %) ventricular ulcer One patient had both forms simultaneously No noticeable differences in body build or age were found between the duodenal and ventricular ulcer patients

On admission to hospital 82 2 % of all ulcer cases had some form of haemorrhage with lowered Hb concentration to on average 8 7 g 100 ml Haemorrhage was more common among duodenal (89 1 %) than among ventricular ulcer patients (62 5 %)

The average period in hospital was 14 days and was as long for those with duodenal as with ventricular ulcer The period of hospitalization was not affected by the fact that some patients bled so much as to require blood transfusions Eight (12 6 %) of the 63 patients were operated on, three of whom (4 7 %) in the acute phase All patients came through their operations satisfactorily

Forty one (65 0 %) of the patients considered they had been under mental stress before the attack An equally large number (65 0 %) had earlier had gastric ulcer No patient had earlier had myocardial infarction Eighteen (28 5 %) had had a parent with gastric ulcer, and four (6 3 %) a parent with myocardial infarction

The mean cholesterol (249 0 mg/100 ml) and triglyceride levels (98 6 mg/100 ml) in serum were both normal Only nine patients (14 1 %) stated that they kept a light ulcer diet These did not differ from the remainder as regards blood fat concentration in serum No significant correlation was found between cholesterol and triglycerides on the one hand and age, weight, height, femoral condyle width and skinfold thickness on the other

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Organ-specific and Organ-non-specific Auto-antibodies in Rheumatoid Arthritis

By

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The object of the present report was to study the occurrence of some auto-antibodies in rheumatoid arthritis and to examine their mutual correlations, if any The reactions involved were tests for rheumatoid factors, tests for antinuclear factors and tests for some organ specific auto-antibodies (thyroid gastric parietal cell adreno-cortical and parotic duct antibodies) Smooth muscle antibodies and mitochondria antibodies supposed to be tracers for a liver cell damage (5) were also determined, furthermore, some classic reactions for demonstrating infection were included

Material and methods

Sera from 100 patients with classic or definite rheumatoid arthritis according to the ARA criteria (4) treated as in patients in the King Christian X Arthritis Sanatorium at Grasten, were examined

The control sera were drawn from 100 healthy blood donors matched for age and sex

Owing to limited amounts of sera some reactions were performed in only a lesser number of sera

Rheumatoid factors were determined 1 by the sensitized sheep-cell agglutination test (Waaler Rose reaction) in a modification described by Bichel et al (3) 2 by means of the streptococcal L agglutination reaction (SA) described by Kalbak (16) and 3 using latex particles sensitized with human gamma globulin (Hyland reagent)

Antinuclear factors (ANF) The test for ANF was performed as described (6 8) using the following human tissues as nuclear antigen in the indirect fluorescent antibody technique human blood smears altered by repeated freezing and thawing human thyrotoxic gland human gastric mucosa human kidney tissue and human striated muscle All solid tissues were obtained at operations and immediately after removal were cut into pieces and frozen.

Thyroid antibodies Antibodies against thyroglobulin were determined by a passive haemagglutination technique described by Fulthorpe et al (11) while antibodies against the microsomal antigen were determined by the fluorescent antibody technique employing thyrotoxic gland as antigen The organ specificity was ascertained by simultaneous examination of the reaction of sera

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with cytoplasmic substances in human tubular cells

The indirect fluorescent antibody technique was used for the determination of the following antibodies

Parietal cell antibodies, parotic duct antibodies adreno cortical antibodies mitochondria antibodies and smooth muscle antibodies Human gastric mucosa, human kidney and parotic gland and adrenal gland from a guenon monkey were used as antigen The optimal reacting tissue for mitochondria antibodies was human tubular kidney cells but this antibody also shows a characteristic granular fluorescence with cytoplasmic fractions in the other human tissues used Smooth muscle antibodies were determined by use of the lamina muscularis in the gastric tissue

Wassermann reaction (WR) the gonococcus complement fixation test (GR) the anti streptolysin-O test (ASL) and the anti streptococcal hyaluronidase test (ASH) were performed by the standard methods of Statens Seruminstitut

Performance of tests interpretation of results and reproducibility

In the fluorescent antibody technique the sera were used undiluted they were incubated with the antigen for 30 minutes and after repeated washing in buffered saline, the tissue sections were incubated with FICT labelled anti human gamma globulin Most tests were repeated twice or more if the results were doubtful The interpretation of the tests for mitochondria antibodies thyroid cytoplasmic parietal cell parotic duct and adrenal cortical antibodies was as follows fluorescence of cytoplasmic substances in parietal cells thyroid epithelial cells adrenal cortical cells and parotic duct cells with no fluorescence of kidney tubular cells was considered indicative of the presence of organ specific antibodies against the antigens mentioned Mitochondria antibodies were considered present when the sera showed fluorescence of cytoplasmic substances in kidney tubular cells and in epithelial cells of gastric mucosa thyroid gland and parotic gland Furthermore, the mitochondria antibody frequently gave rise to a characteristic granular fluorescence

Results

The results appear from tables I—V and figs 1—2 The incidence of the different serological reactions in the pathological and control sera is shown in table I, the distribution of the different results according to sex and age is shown in table I and figs 1—2, while the mutual relationship of the factors is shown in tables II—V

Rheumatoid factors In a titre of ⩾ 1 20 the FII sensitized latex test was positive in 82 % of the sera from patients with rheumatoid arthritis, but also in 18 % of a control material consisting of normal, healthy persons, matched for age and sex, a finding which stresses the sensitivity, but also the non specificity of this test

A positive sensitized sheep cell agglutination test (SSCT) in a titre of 1 20 or greater was found in 2/3 of the sera and in 6 % of the controls If a dilution of 1 40 was used, only one of the normal control sera was positive, but the incidence of positive reactions in sera from patients with rheumatoid arthritis then decreased to 54 %

The streptococcal agglutination test (SA) was positive in 48 % of the sera but only in sera showing either a positive sheep cell agglutination test or a positive latex test

Antinuclear factors The highest incidence of ANF was found when polymorphonuclear granulocytes were used as antigen Thus granulocyte reactive

TABLE I The incidence of organ specific and organ non specific auto-antibodies rheumatoid factors and antibacterial antibodies in sera from patients with rheumatoid arthritis

	No of pats	Number of positive sera					
		♀	♂	♀+♂	%	Controls[1]	%
Rheumatoid factors							
FII sensitized latex test > 1 20	100	64	18	82	82	18	18
Streptococ aggl test	100	36	12	48	48	3	3
Sheep-cell aggl test > 1 20	100	51	15	66	66	6	6
> 1 40	100	40	14	54	54	1	1
> 1 80	100	32	10	42	42	0	0
Antinuclear factors (ANF)							
Granulocyte reactive	84	43	13	56	66	5	6
Lymphocyte reactive	84	15	5	20	24	5	6
Thyroid reactive	84	19	3	22	26	5	6
Gastric mucosa reactive	77	6	1	7	9	3	4
Kidney reactive	77	5	2	7	9	6	8
Striated muscle reactive	77	2	0	2	2 5	2	3
Organ specific antibodies							
Thyroid antibodies							
thyroglobulin ≥ 1 5	77	6	3	9	11	10	11
cytoplasmic	77	3	0	3	3	12	15
Parietal-cell antibodies	77	1	0	1	1	2	2
Parotic duct antibodies	77	8	4	12	15	0	0
Adreno-cortical antibodies	77	1	0	1	1	1	1
Smooth muscle antibodies	77	4	2	6	7	6	7
Mitochondria antibodies	77	7	0	7	7	7	7
Antibacterial antibodies							
Wassermann reaction	100	2	0	2	2	—	—
Gonococcus compl fix test	100	0	0	0	0	—	—
Antistreptolysin O test	100	6	1	7	7	12	12
Antistreptococ hyaluronidase	100	9	2	11	11	4	4
Total numbers of patients	100	79	21			100	

[1] Matched for age and sex

ANF was present in almost 2/3 of the sera (56 of 84 sera tested) This should be compared with thyroid reactive and lymphocyte reactive ANF being present in only 1/4 of the sera, gastric mucosa reactive and kidney reactive ANF in seven of the investigated sera (8.9 %) and ANF reactive with human striated muscle in two sera only Although the granulocytes show a very high reactivity with sera from patients with rheumatoid arthritis nuclear reactivity was found in only 6 % of normal sera a finding not incompatible with the prevalence of ANF in the controls when other tissue nuclei were used

Thus while approximately 2/3 of sera with a latex test titre less than 1 160 showed a positive ANF reaction with granulocytes, almost 95 % of sera with a higher latex titre showed positive reactions for granulocyte reactive ANF

The two factors do, however, occur in sera independently of each other, since almost 1/4 of sera with a positive latex reaction did not contain ANF and five sera showing a negative latex test did contain granulocyte reactive ANF

Sensitized sheep-cell agglutination test and ANF (tables III and V) Although half of the sera with a negative SSCT test showed a positive reaction for ANF, almost 3/4 of the sera with a positive SSCT test showed a positive reaction for ANF also the difference is, however, not statistically significant at the 5 % level and there was no significant increase in the incidence of granulocyte-reactive ANF with increasing SSCT titre

There was no significant difference between the incidence and titre of the two rheumatoid factors and ANF with mono- or multinuclear reactivity

Discussion

In the present study a number of serological tests detecting the presence of a broad spectrum of tissue antibodies anti gamma globulin and antibacterial antibodies were performed using sera from patients with rheumatoid arthritis of various degrees of severity and duration The results show, however that only rheumatoid factors and ANF are found significantly more frequently when compared with the incidence in sera from normal controls, matched for age and sex Thus, there seems to be no serological overlapping of rheumatoid arthritis and auto-immune diseases characterized by the presence of organ specific auto-antibodies This is rather remarkable in view of the serological and clinical overlap often noticed in other auto-immune diseases, such as thyroiditis, pernicious anaemia and adreno cortical deficiency (10) Clinical manifest kerato-conjunctivitis sicca was found in two cases only, and systematic parotic gland biopsies are probably needed in order to reveal directly whether the finding of parotic gland antibodies in 12 patients shows that they are tracers indicating a parotic gland damage No liver specific auto antibodies have been described previously However, antibodies against mitochondria and smooth muscle have hitherto especially been found in patients with chronic liver cell damage (5), particularly biliary cirrhosis (19) and lupoid hepatitis (15) The present finding of liver auto-antibodies in approximately 10 % of the sera is in keeping with the result obtained by Doniach et al (5) Since the usual liver laboratory tests are of questionable value in assessing a liver cell damage in rheumatoid arthritis, it is doubtful whether the presence of these two auto-antibodies in some sera indicates a liver cell damage in these patients, and no liver biopsy was performed

The frequent presence of antinuclear factors — as determined by the immunofluorescent technique—in sera from patients with rheumatoid arthritis has been the subject of several reports The

prevalence has been found to vary from 6 to 70 %. This finding has been chiefly attributed to variations in the sensitivity of the technique or to differences in the composition of the material. However, recent reports have shown that ANF can be found to react selectively with polymorphonuclear granulocytes (7) and that such ANF are found almost exclusively in rheumatoid arthritis (9). The literature also reveals that ANF reacting with polymorphonuclear granulocytes have actually been found to be very highly prevalent in rheumatoid arthritis (1, 13). The systematic investigation here reported confirms previous reports (2, 6 10) that the reactivity of ANF in rheumatoid arthritis largely depends on the nuclear substrate used as antigen. The mutual relationship of these ANF and the clinical and biological significance of granulocyte reactive ANF of different immuno-globulin classes were the subject of the accompanying article (6 a). As regards ANF which are not granulocyte *specific* i e ANF reactive with human thyroid nuclei gastric mucosa, human kidney and human granulocytes and lymphocytes, these antibodies show no significant correlations to sex and age of patients or to clinical parameters such as stage functional class and duration of disease nor to thymol turbidity ESR or concentration of haemoglobulin. These ANF were significantly correlated only to the presence of nodules.

ANF have been found to combine with rheumatoid factors in some cases (2 14). The report of McCormick and Day (17) suggests a nuclear specificity of some rheumatoid factors. Most reports (2, 12, 18), however, maintain that ANF and rheumatoid factors are separable antibodies. The results of the present report point to the same conclusion. Thus, although most sera with a positive test for rheumatoid factors also reacted in the ANF test, ANF were also found in more than half of the sera from patients with a negative SSCT test and in almost 1/3 of the sera from patients with a negative latex test. The latex test is probably the first of these two tests to be positive and the most frequently positive test in the first five years. Thus, in patients with duration of disease less than five years, approximately 1/3 of the sera showing a positive latex test were negative in the ANF reaction, while only two ANF positive sera in this group of patients were negative in the latex test. This sequence is, however, not so conspicuous in the group of seven patients with disease of very short duration (< 1 year) where five were positive in the ANF and latex tests and two were entirely negative.

In patients with disease of longer duration ANF and rheumatoid factors are more frequently found together i e while 1/4 of patients with disease of short duration showed a positive latex test, but a negative ANF test this serological pattern was found in only 1 9 of patients with disease of longer duration.

The incidence of a positive ANF test is approximately the same as that of a positive SSCT test. There was no significant difference in their mutual relation ship as regards duration of disease and these two serological tests were independent of each other. Thus in the group of patients with disease lasting

less than five years, sera from seven patients showed a positive ANF but a negative SSCT test, eight patients showed a negative ANF but a positive SSCT test, almost identical patterns were found in patients with more long lasting disease In the group of patients with disease of less than one year's duration, five out of seven sera showed granulocyte *specific* ANF and none of these showed a positive SSCT reaction However, the data are too scanty to give any clear information about the chronological relationship between these two factors

Summary

One hundred sera from patients with rheumatoid arthritis were investigated for the presence of a broad spectrum of tissue antibodies, anti gamma globulin and antibacterial antibodies including 1 *rheumatoid factors* determined by the FII sensitized latex test, the sensitized sheep cell agglutination test (SSCT) and the streptococcal agglutination test (SA) 2 *ANF* determined by the fluorescent antibody technique with different human tissues as nuclear antigens, 3 *tissue auto antibodies* parotic duct anti bodies parietal cell antibodies thyroid antibodies adreno cortical antibodies smooth muscle and mitochondria anti bodies Furthermore some tests for anti bacterial antibodies were performed

Organ specific tissue antibodies were not found to be significantly more frequent in sera from rheumatoid arthritis than in sera from healthy normal persons matched for age and sex

The FII latex test (≥ 1 20) was positive in 82 %, the SCCT test (≥ 1 20) in 2/3 of the sera, while the SA test was positive in only 48 %—always in sera showing a positive latex or SSCT test

ANF reactive with human granulocytes were found in 2/3 of the sera, while ANF reactive with other human nuclei were present in from 25 % (thyroid reactive and lymphocyte reactive) to 10 % (human gastric mucosa, human kidney nuclei) With human striated muscle only 2 % of the sera showed a positive ANF reaction

The relationship between ANF and rheumatoid factors is discussed in the light of a significant correlation between antihuman gamma globulin factors and granulocyte reactive ANF, with special emphasis on the occurrence of these factors in relation to duration of disease

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Lipid Mobilization from Human Subcutaneous Adipose Tissue in Vitro

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In the rat epididymal fat pad the utilization of glycerol is low, and usually the glycerol produced originates from the complete cleavage of triglycerides (24) Glycerol production is therefore probably a good measure of adipose tissue triglyceride hydrolysis (24)

These reactions are only partially know in human adipose tissue Glycerol utilization during basal conditions is low (14) Glycerol is released, but the extent to which it originates from the complete hydrolysis of triglycerides partial glycerides or even from non glyceride sources is not known Furthermore, the lipase activity which is believed to be mainly responsible for triglyceride hydrolysis in rat adipose tissue (5 13, 18, 25) has only been qualitatively described in human adipose tissue (1)

Studies of these reactions in human adipose tissue seem worthwhile not only because species differences of adipose tissue metabolism have been repeatedly noticed (8 21) but also because classification is needed of the abnormally elevated glycerol release from subcutaneous adipose tissue in human diabetes mellitus described previously (6, 27)

In the present work these reactions have been studied in human subcutaneous adipose tissue and compared with corresponding reactions in the rat epididymal fat pad

Material and methods

Human subcutaneous adipose tissue was obtained from patients operated on for different abdominal diseases They were fasting for 18 hours before operation The specimen was taken from the upper part of the abdominal subcutaneous fat layer placed in Krebs Ringer bicarbonate buffer pH 7 4 at room temperature brought to the laboratory and immediately processed

Rat epididymal fat pads from 250—350 g Sprague Dawley rats fed ad libitum were obtained as previously described (3)

Total adipose tissue glycerol and free fatty acid production were determined in incubation systems consisting of 3 ml Krebs-Ringer bicarbonate buffer containing 4 % serum al

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bumin (Bovine serum albumin Fraction V, Armour batch KC 2271) Glucose was present at a concentration of 10 mM Samples of human or rat tissue (100—200 mg) in duplicate flasks were first incubated for 30 min to allow equilibration between the medium and tissue glycerol From the medium the first aliquots for glycerol (15) and fatty acid determinations (2, 9) were then taken and the tissues from two flasks taken for the determination of tissue fatty acid concentration Thereafter additions of insulin (Crystalline pig plus bovine insulin Nordisk Insulin, Gentofte Denmark) (10 000 μU/ml) or norepinephrine (50 μg/ml) were made and incubation continued for another 60 min The concentrations of glycerol and fatty acids in the medium and fatty acid concentration in the tissues were then determined again

In experiments where glycerol utilization was measured, three pieces of human adipose tissue, each weighing about 200 mg, were incubated as described above but without glucose The final free fatty acid concentration of this incubation medium was 0 15 mM Glycerol was added to all flasks to a final concentration of 5 mM and approximately 200 000 counts/min of 1 ^{14}C Glycerol (The Radiochemical Centre Amersham England CFA47) Two flasks were incubated without further addition and two flasks with 10 000 μU/ml of insulin In a similar series run in parallel the albumin was prepared so that the final incubation medium contained a concentration of 0 85 mM of free fatty acids This was accomplished by the addition to a 20% albumin solution, with thorough mixing of a suspension of palmitic acid in water brought to pH 7 4 with KOH Incubations were performed in 50 ml cylindrical flat bottom tubes oscillating 120 times/min in an incubation bath at 37 0° C The final pH was 7 4 and the gas phase was 95 % O_2 and 5 % CO_2 Radioactivity in carbon dioxide, fatty acids and glyceride glycerol was determined as described previously (4) and calculated as glycerol from the initial specific activity of the incubation medium and the counts obtained

When incorporation of glucose into glyceride glycerol and free glycerol was measured, the incubation medium was the same as that described with addition of glucose to a final concentration of 10 mM and also approximately 200,000 counts/min of U ^{14}C glucose (The Radiochemical Centre Amersham England CFB35) After incubation for 60 min glyceride glycerol radioactivity was determined, 1 ml of the medium added to 1 ml acetone and the protein precipitate centrifuged Aliquots of the supernatant were applied on silicic acid thin layer chromatoplates (Kieselgel nach Stahl, Merck Darmstadt Germany) and run in chloroform acetone 5N ammonia (10 80 10) (20) until a suitable running distance was obtained Glycerol and glucose were run separately as standards Duplicate aliquots from an incubation medium and standards were run on each plate After running for a suitable distance the plates were dried at about 100° C until all smell of ammonia had disappeared The area of the plate on which standards had been applied was sprayed with developing agents (20) with the other part of the plate covered Areas corresponding to glycerol (R_f = 0 3—0 4) and glucose (R_f = 0 1) were then scraped into counting vials and counted as described by Snyder and Stephens (19)

Lipase activity was determined as described previously (2) Human adipose tissue needed to be homogenized in homogenizers with a somewhat loosely fitting pestle (1) The whole homogenate was assayed Since the amount of the endogenous lipids was sufficient to give saturation no addition of substrate was made unless stated otherwise

In experiments where hydrolysis of partial glycerides was measured adipose tissue was taken directly after excision and homogenized with 2 ml 0 15 M potassium chloride and 0 1 g of glycerylmonooleate (Hopkins and Williams) or 0 1 g of glycerylmonostearate (BDH) or 0 1 g of glyceryl 1 2 dipalmitate (melting point 59 2—60 5°) or as a control in each series of experiments, 0 1 g of human adipose tissue lipids These were obtained by extraction of human adipose tissue in

TABLE I Balance between fatty acid and glycerol production in human and rat adipose tissue

	Human n = 8	Rat n = 8
Tissue fatty acid concentrations		
0[1] (μEq/g)	1 15±0 04	3 28±0 20
60 (μEq/g)	1 02±0 05	1 48±0 20
60 minus 0 (μEq/g)	−0 13±0 04	−1 80±0 20
Medium fatty acid concentration		
60 minus 0 (μEq/g)	1 18±0 08	1 27±0 18
Total fatty acid production		
(μEq/g per 60)	1 05±0 07	−0 53 ±0 21
Glycerol production		
(μmoles/g per 60)	0 37±0 10	1 01±0 08
Fatty acids "reesterified "[2] (μEq/g per 60)	0 06±0 15	3 56±0 21

Means ± S E M

[1] Times given are after 30 preincubation

[2] Calculated as glycerol production × 3 minus total fatty acid production (cf (24) and Discussion)

methanol chloroform partition by the method of Folch et al (10) and evaporation of the chloroform phase under nitrogen. One ml of the homogenate 0 2 ml of 20 % albumin pH 7 0 and 0 1 ml of 0 25 M phosphate buffer pH 7 0 were then incubated and the free fatty acid concentration determined at 0 and 30 min. All flasks were incubated in duplicate

Results

Balance of glycerol and fatty acid production

Balance of glycerol and fatty acid production measured essentially in accordance with Vaughan (24), is shown in table I

In the basal state total fatty acid production was higher in human than in rat adipose tissue Rat epididymal fat pads showed a net uptake of fatty acids in spite of the fact that glycerol production was higher in these tissues than in human tissues Insulin in a high dose caused a decrease in net fatty acid production as seen in table II and in some cases a net uptake could be demonstrated Glycerol release appeared to be unchanged When norepinephrine was added glycerol and free fatty acid production were increased

Metabolism of glycerol

In order to explain the higher total fatty acid production in relation to glycerol release in human adipose tissue and its lower glycerol production factors determining the turnover of the free glycerol pool were studied

Glycerol utilization was first investigated Table III shows that when the fatty acid concentration of the incubation medium was low a small but significant

TABLE II Balance between fatty acid and glycerol production in human adipose tissue Effects of insulin and nor epinephrine

	No addition	Insulin (10,000 μU/ml)	Nor-epinephrine (50 μg/ml)
Tissue fatty acid concentrations			
[1]0 (μEq/g)	1 09 ± 0 04	1 09 ± 0 04	1 09 ± 0 04
60 (μEq/g)	1 31 ± 0 05	0 85 ± 0 04	2 12 ± 0 10
60 minus 0 (μEq/g)	0 22 ± 0 03	—0 23 ± 0 05	1 03 ± 0 09
Medium fatty acid concentration			
60 minus 0 (μEq/g)	0 86 ± 0 07	0 31 ± 0 07	1 15 ± 0 09
Total fatty acid production			
(μEq/g per 60)	1 08 ± 0 10	0 08 ± 0 06	2 18 ± 0 14
Glycerol production			
(μmoles/g per 60)	0 41 ± 0 04	0 38 ± 0 04	0 71 ± 0 04
Fatty acids reesterified [2]			
(μEq/g per 60)	0 15 ± 0 11	1 06 ± 0 05	—0 05 ± 0 13

Means ± S E M n = 8

[1] Times given are after 30 preincubation

[2] Calculated as glycerol production × 3 minus total fatty acid production (cf (24) and Discussion)

TABLE III Metabolism of 1 ^{14}C-glycerol in human adipose tissue

	CO_2 (mμmoles/g/hr)	Glyceride		Total (mμmoles/g/hr)
		Fatty acids (mμmoles/g/hr)	Glycerol (mμmoles/g/hr)	
Low FFA in medium				
0	12 4 ± 0 7	36 5 ± 4 4	17 8 ± 4 8	66 7 ± 5 9
Insulin (10 000 μU ml)	10 9 ± 0 9	35 5 ± 4 2	16 2 ± 4 2	62 6 ± 5 1
High FFA in medium				
0	19 4 ± 2 9[1]	45 0 ± 7 3	17 4 ± 5 3	81 8 ± 6 1[3]
Insulin (10 000 μU/ml)	*20 8 ± 1 9[2]*	*36 5 ± 4 4*	*23 4 ± 3 9*	*80 7 ± 4 7[3]*

Means ± S E M n = 4

Low FFA concentration 0 15 mM

High FFA concentration 0 85 mM

All comparisons with low FFA in medium 0

[1] p<0 10>0 05

[2] p<0 05

[3] p<0 02

amount of glycerol from the incubation medium was incorporated into the measured metabolites As reported previously (14) this amount is about 1/10 of the incorporation from 1 ^{14}C glucose under similar conditions With higher fatty acid concentrations in the medium, incorporation into carbon dioxide was almost doubled while labelled fatty acid and glyceride glycerol did not change Insulin had no influence

Non lipolytic glycerol release

C 14 from glucose was incorporated into glyceride glycerol while no significant incorporation into the released free glycerol could be found These results accord with those obtained in the rat (7) and assuming a small α glycerolphospate pool in human adipose tissue, provide evidence that in human adipose tissue, glycerol cannot be released directly from the α glycerolphosphate pool

Lipolytic activity in homogenates

Since it had been shown previously that the hormone sensitive lipase of the rat epididymal fat pad can be found in human adipose tissue (1) an assay system optimal for this type of activity was used (25) These results are given in table IV Glycerol release was approximately doubled by the addition of a large amount of norepinephrine and addition of ascorbic acid to avoid oxidation of norepinephrine caused only a small further increase Lipase activity increased slightly but significantly after norepinephrine with or without ascorbic acid The activity was small compared with that in the rat (3)

TABLE IV Nor epinephrine activation of glycerol release and lipase activity in human subcutaneous adipose tissue

Addition to incubation mixture	Glycerol release[1] (μmoles/g/hr)	Lipase activity (μEq FFA/g/30)
0	1 04	0 30
Nor epinephrine (50 μg)	2 36	0 39
Nor epinephrine (50 μg) + ascorbic acid (2 5 μmole)	2 47	0 45

Means n = 3

[1] Measured without 30 min pre incubation

The discrepancy between these results from human adipose tissue and those obtained with rat adipose tissue prompted a direct comparison between these two Such a comparison at different times after removal of the tissues, is given in fig 1 In the rat, lipase activity and glycerol release tended to decrease after removal of the tissues and then increased by a factor of 2—3 after norepinephrine addition These parameters seemed to follow each other but on different activity levels as previously described (3) In the human tissues glycerol release was somewhat lower than in the rat tissues A twofold increase was found after norepinephrine stimulation Lipase activity was low and amounted to only about 1/5 of that in the rat This difference was still more pronounced after the small norepinephrine stimulation of human lipase activity which was only about 1/10 of that in the rat

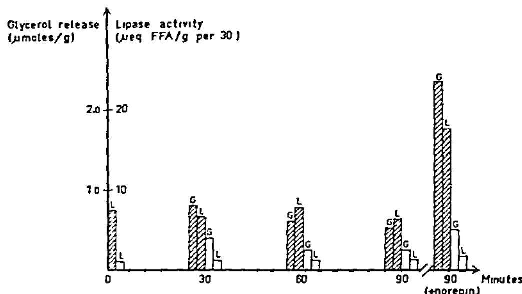


Fig 1 Glycerol release from and lipase activity in human (open) and rat (striped) adipose tissue at different times after incubation Values are means of three experiments Norepinephrine (50 µg/ml) added at O G = glycerol release L = lipase activity

Hydrolysis of partial glycerides

The higher ratio of net fatty acid production/glycerol release in human than in rat adipose tissue (table I) could be explained by qualitative differences between the lipolytic process in human and rat adipose tissue Hydrolysis of triglycerides to partial glycerides would thus be expected to produce such a high ratio Therefore the ratio of fatty acid pro-

TABLE V Fatty acid and glycerol release in homogenates from human subcutaneous adipose tissue and rat epididymal fat pad

	Human	Rat
Fatty acid release (µEq g 30)	0 40 ± 0 08	4 27 ± 0 41
Glycerol release (µmoles g 30	0 15 ± 0 03	1 54 ± 0 21
Fatty acid release / Glycerol release	2 7	2 8

Means ± S E M n = 6

TABLE VI Lipase activity in human subcutaneous adipose tissue homogenate after addition of different glycerides

	Fatty acids released (µEq/g/30)
Human adipose tissue triglycerides (0 1 g)	0 43 ± 0 04
Monoolein (0 1 g)	10 0 ± 1 2
Monostearin (0 1 g)	4 24 ± 0 8
1 2-dipalmitin (0 1 g)	1 04 ± 0 06

Means ± S E M n = 6

Summary

Lipid mobilization from the rat epididymal fat pad is known in considerable detail while the reactions responsible for lipid mobilization in human subcutaneous adipose tissue are only partially known

A comparison between the balance of glycerol and free fatty acid production in human and rat adipose tissue revealed that the ratio of free fatty acid production/glycerol production was higher in the human tissues Norepinephrine increased this ratio in human tissues while insulin decreased it

The reason for this discrepancy was investigated It was found that glycerol release is probably a measure of lipolysis in human tissue as it is in rat adipose tissue It was found that glycerol released from human subcutaneous adipose tissue probably riginate directly from

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fore considered to be due mainly to a relative incapacity of the re-esterification system of adipose tissue from fasting humans as compared with that in rat tissue The possibility of accumulation of partial glycerides in human adipose tissue during the lipolytic process in certain conditions was also considered

The results indicate a lower turnover rate of adipose tissue triglycerides in the human than in the rat implying perhaps a more static fat depot in the human

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shown, however, that radioactive fatty acids are readily incorporated into the glycerides of human adipose tissue in vitro. Even if such techniques do not seem to allow quantitation of the re-esterification process, due to unknown isotope dilution (26), Östman's (27) data taken together with those of table I probably indicate that an unknown amount of fatty acids was released without glycerol production. Although indirect this seems to be an indication of accumulation of partial glycerides in human adipose tissue during lipolysis. The still higher ratio of fatty acid production/glycerol release during nor-epinephrine stimulation (table II), found independently by Martinsson (16) could be an indication of further accumulation of partial glycerides during these conditions.

The patients from whom the adipose tissue was taken were fasting for about 18 hours but the rats were fed ad libitum. This could mean a decreased re-esterification in the human tissues as compared with tissues from non-fasting subjects. Comparisons with the rat tissues are still pertinent since Vaughan (24) has shown that even if the rats had been fasted as long as these patients re-esterification would still not have been impaired.

Recent data presented by Havel (11) indicate a ratio of fatty acid turnover rate/glycerol turnover rate in plasma higher than three in the human particularly during exercise with increased lipid mobilization. These data accord with the findings in tables I and II and seem to strengthen the validity of these data for in vivo conditions.

In human adipose tissue the possible contribution of free fatty acids from partial hydrolysis of triglycerides could be very small. In this case a re-esterification deficiency would be relatively large since only a small part of the released fatty acids had then been re-esterified (cf. table I). If partial hydrolysis of triglycerides made a very large contribution to the free fatty acid pool, the re-esterification process would also be insufficient to retain the free fatty acids within the adipose tissue. Human adipose tissue lipid mobilization therefore seems to be more influenced by a deficient capacity of the re-esterification process than that in rat adipose tissue. Re-esterification in the rat thus keeps pace with lipolysis after fairly long fasting (24) and α-glycerolphosphate synthesis is not decreased early in uncontrolled alloxan diabetes (23). Lipolytic activity in human adipose tissue is also low as compared with the rat, as reflected by the low glycerol and fatty acid release after lipolytic hormones (17) and the low lipase activity in homogenates. This could mean that human lipid mobilization depends not only on lipolysis but also to some degree on restricted re-esterification, while in the rat the lipolysis predominates.

The probably lower rate of glyceride breakdown in human adipose tissue as compared with that in the rat epididymal fat pad and also the probably lower rate of re-esterification in human tissues indicate a lower turnover rate of human adipose tissue triglycerides. This implies a more static subcutaneous fat depot in the human as compared with the rat epididymal fat pad.

Summary

Lipid mobilization from the rat epididymal fat pad is known in considerable detail, while the reactions responsible for lipid mobilization in human subcutaneous adipose tissue are only partially known

A comparison between the balance of glycerol and free fatty acid production in human and rat adipose tissue revealed that the ratio of free fatty acid production/glycerol production was higher in the human tissues Norepinephrine increased this ratio in human tissues while insulin decreased it

The reason for this discrepancy was investigated It was found that glycerol release is probably a measure of lipolysis in human tissue as it is in rat adipose tissue It was found that glycerol released from human subcutaneous adipose tissue probably did not originate directly from the α glycerolphosphate pool and that utilization of free glycerol was insignificant

The ratio free fatty acid release/glycerol release was the same in human and in rat adipose tissue homogenates suggesting that partial glycerides do not accumulate more in human adipose tissue than in rat adipose tissue Furthermore, lipolytic activity against partial glycerides was high in human adipose tissue homogenates These results taken together with previous data showing that no partial glycerides are present in human adipose tissue, do not suggest the accumulation of partial glycerides in human adipose tissue during the lipolytic process

The high ratio of free fatty acid production/glycerol production was therefore considered to be due mainly to a relative incapacity of the re esterification system of adipose tissue from fasting humans as compared with that in rat tissue The possibility of accumulation of partial glycerides in human adipose tissue during the lipolytic process in certain conditions was also considered

The results indicate a lower turnover rate of adipose tissue triglycerides in the human than in the rat implying perhaps a more static fat depot in the human

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Serum Lipids in Male Patients Hospitalized for Myocardial Infarction

By

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Several authors have shown that patients with coronary heart disease usually have elevated serum lipid levels (1 2, 6, 8, 12) The study from Framingham by Drawber et al (12) showed that the combination of hypercholesterolaemia, hypertony and adiposity in middle aged men involves an especially high risk Each second person with this triad had symptoms of coronary heart disease against 1 in 25 among the remaining population Biorck et al (2), in a study from 1957, showed that high cholesterol levels occurred particularly in men with myocardial infarction in the 40—49 age group Carlson (8), in a study published in 1960 showed that patients attacked by myocardial infarction before the age of 50 had high triglyceride levels while especially the cholesterol concentration in serum was high if the infarction came later In 1966 Biorck et al (6) showed that coronary heart disease is more common in younger men with hypercholesterolaemia and in men with high blood pressure A relation, though not proved, thus appears to exist between elevated serum lipids and the development of myocardial infarction

Whether male patients treated at the largest hospital in Stockholm County on a diagnosis of myocardial infarction have high cholesterol and/or triglycerides in serum one year after discharge has not been investigated in recent years This has therefore been specially studied in the present investigation

Since several authors (23, 24, 25) in postmortem examinations have found that peptic ulcer patients appear to run a greater risk than others of death from myocardial infarction the number of patients with X ray verified peptic ulcer is reported in the present study

Material

The material consists of 110 male patients hospitalized during 1965 in the Medical Clinic of Danderyd Hospital on a diagnosis

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of myocardial infarction In all cases the diagnosis was based on a typical clinical course verified by ECG and elevated GOT Of these 110 patients 27 died during hospitalization A special account of these will be given Of the remainder two had had diabetes mellitus and were excluded from the survey Of the remaining 81 discharged cases six died before the follow up could be made Finally there were two patients who for different reasons could not be got to come for examination Thus of the 81 discharged patients 73 (90 1 %) were followed up It should be pointed out that all patients were from the hospital s own reception area with a population of rather more than 200 000 in the northern part of Stockholm County and not from the remainder of the country

Methods

Records were made of height weight, blood pressure (diastolic pressure at the moment when the Korotkow sounds rapidly diminished in loudness) and age Measurements were made of the skinfold thickness on the medioclavicular line at the level of the navel and of the femoral condyle width in the right knee at an angle of 90° by the method reported earlier (21) The patients heart volumes were recorded per square metre of body area measured with the patient standing as reported earlier (16 20 22)

GOT (glutamic oxalacetic acid transaminase) was determined in Karmen units as previously reported (18) The highest value of GOT measured for each patient was recorded

The blood samples were collected in the morning on a fasting stomach Determinations were made of cholesterol and triglyceride levels in serum by the earlier described method (7 9) and also of the sedimentation rate white count haemoglobin concentration serum iron and transferrin The urine was also analysed for protein and sugar

Every patient was personally questioned by the examiner as to whether he particularly avoided milk, butter and fat food and whether he took nicotinic acid drugs or not also whether he had previously had a myocardial infarction or peptic ulcer and in such case when The patients were asked too whether they considered they were suffering particularly from mental stress at the time of onset either because of work or for other reason and if so whether the stress was so acute that it might be considered to have been a cause of their illness Notes were made of the period in hospital Finally all patients were asked whether either parent had had peptic ulcer or myocardial infarction

Results

Table I shows the age, body dimensions, laboratory tests, length of hospitalization and blood pressure of the followed up patients The mean age was 60 years, which agrees well with the age of the subjects studied by Biorck et al (4) As regards body dimensions, the height was so small and the femoral condyle width so great that these values were close to those found by Hellstrom (15) for weight lifters and wrestlers Compared with these athletes, however, the infarction patients had a greater skinfold thickness and greater weight

The mean levels of cholesterol (286 range 183—459) and triglycerides (177 range 42—517) were higher than an earlier study (7) showed to be normal for men of this age No significant correlation was found between on the one hand cholesterol and triglycerides and on the other age, weight height femoral condyle width and skinfold thickness

A study has been made of the distribution of cholesterol and triglycerides in the material Table II shows the values for different age groups The significantly highest cholesterol levels

TABLE I Values recorded for the patients at follow up one year after hospitalization for myocardial infarction

	n	$\bar{x}$	SD	e_x
Age years	73	60 6	9 6	1 1
Height, cm	73	173 3	6 2	0 7
Femoral condyle width cm	73	10 14	0 41	0 05
Skinfold thickness mm	73	23 8	7 0	0 8
Body weight kg	73	76 6	9 9	1 2
Systolic B P mm Hg	73	153 2	25 3	3 0
Diastolic B P mm Hg	73	97 1	13 8	1 6
Cholesterol mg/100 ml	73	285 6	53 6	6 3
Triglycerides mg/100 ml	73	*177 4*	*109 8*	*12 9*
Sedimentation rate mm/hr	73	14 8	18 6	2 2
White blood cells/mm	73	6 260	1 992	233
Hb concentration g/100 ml	73	14 6	1 2	0 1
Serum iron gamma %	73	115 8	39 9	4 7
Transferrin gamma %	73	334 0	51 9	6 1
GOT Karmen units	68	135 7	74 5	9 0
Heart volume ml/sq m	73	493	77	9
Time in hospital days	73	22 6	8 8	1 0

were recorded for the youngest age groups No significant difference between the groups was found however, as regards triglycerides The number of patients was also found to be evenly distributed between the groups The patients have been classified on the basis of cholesterol and triglyceride levels (table III), as *had been done in earlier* studies (13 17) It will be seen that 30 patients (41 0 %) had normal cholesterol as well as triglyceride levels Of these *30* patients table IV shows that 9 (12 3 % of the entire material) had excessively low cholesterol and triglyceride levels without traceable symptoms of high metabolism Table III shows, furthermore, that 10 subjects (15 0 %) had high cholesterol with normal triglycerides 15 (20 5 %) high triglycerides with normal cholesterol, and 18 (24 6 %) high levels both of cholesterol and triglycerides Of these 18 patients 12 (16 4 % of the entire material) had excessively high cholesterol and triglycerides (table IV) All in all therefore 59 % of the infarction patients had high blood fat values Finally as regards the mean age of the various groups (table

TABLE II Mean cholesterol and triglyceride concentrations in different age groups

Age	n	Mean cholesterol *(mg/100 ml)*	Mean triglycerides *(mg/100 ml)*
40—55	25	296	183
56—65	23	293	156
66—79	25	*268*	184

TABLE III High and low values of cholesterol and/or triglycerides

Serum lipids (mg/100 ml)		n	Age	Mean cholesterol	Mean triglycerides
Cholesterol Triglycerides	<300 <150	30	62 2	250	102
Cholesterol Triglycerides	>300 <150	10	61 7	323	107
Cholesterol Triglycerides	<300 >150	15	60 6	258	261
Cholesterol Triglycerides	>300 >150	18	57 4	347	272

TABLE IV Patients with excessively low and high values of cholesterol and triglycerides

Serum lipids (mg/100 ml)		n	Age	Mean cholesterol	Mean triglycerides
Cholesterol Triglycerides	<250 100	9	67 0	220	82
Cholesterol Triglycerides	>300 200	12	57 7	346	319

III) low blood fat values were found in the older and high in the younger patients The age differences in this respect however were not significant in any case

Table I shows that the mean heart volume in standing position was 493 ml/sq m body area (range 325—670) which is very slightly below the mean (approx 500 ml) considered to represent the upper limit of normal volume in adult men (16 20 22) In no less than 27 cases (37 0 %) the heart volume was clearly enlarged

The mean blood pressure was 153 mm Hg systolic (range 110—210) and 97 mm Hg diastolic (range 70—130) Eight patients (10 9 %) had a systolic blood pressure above 180 and 31 (42 5 %) above 150 mm Hg 25 (34 2 %) a diastolic blood pressure above 100 mm Hg

The mean GOT was 135 7 Karmen units (range 45—350) as appears from table I In five cases the GOT values were normal, in all probability because the patients had come in to hospital three or more days after the onset These cases were nevertheless included in the material as their clinical course and ECG were typical of myocardial infarction Judging from the large variation in GOT values the size of the infarctions appears to have varied greatly Thus 24

TABLE V Differences between patients on and not on a diet

	Diet		No diet		Diff	P
	n	$\bar{x}$	n	$\bar{x}$		
Age years	49	60.1	24	61.6	1.5	>0.2
Height cm	49	173.9	24	172.0	1.9	>0.2
Femoral condyle width cm	49	10.12	24	10.20	0.08	>0.2
Body weight kg	49	76.9	24	76.1	0.8	>0.2
Skinfold thickness mm	49	23.5	24	24.5	1.0	>0.2
Systolic B P mm Hg	49	151.7	24	156.3	4.6	>0.2
Diastolic B P mm Hg	49	94.9	24	101.7	6.8	x
Cholesterol mg/100 ml	49	281	24	294	13	>0.2
Triglycerides mg/100 ml	49	176	24	180	4	>0.2

patients (35 %) had GOT values below 100 Karmen units, 30 (44 %) between 100 and 200, and 14 (21 %) above 200 The time in hospital also varied greatly, from 7 to 60 days mean 22.6 days (table I)

No less than 49 patients (67.1 %) stated that they avoided milk butter and as far as possible, fat food The difference between these patients and the other 24 will be seen from table V but was not significant either as regards weight skinfold thickness cholesterol or triglycerides Only in diastolic blood pressure did those who maintained a diet have a probably significantly lower value

In the personal interviews with patients 42 (57.5 %) considered that mental stress had had an active part in their illness The serum lipid levels of these patients prior to onset is unfortunately not known

Of the total of 73 patients 11 (15.1 %) had earlier had an infarction None of these had occurred earlier than 1960 An equal number of patients had earlier had an X ray verified peptic ulcer Eleven patients likewise, had had a parent with myocardial infarction and six a parent with peptic ulcer

Nine (12.3 %) of the patients stated that they were on AP five that they took a blood pressure lowering medicine, but none a blood fat lowering drug

The 27 infarction patients who died in hospital represented 25.5 % of the total of 110 admissions Among the 27 deaths three were found to have diabetes mellitus These cases are excluded from the following account There remain, therefore 24 deaths Mean age weight, height blood pressure on admission and time in hospital are shown in table VI The patients who died were older, weighed less and were taller than the survivors Both systolic (153 mm Hg range 95—280) and diastolic blood pressure (95 mm Hg range 64—140) must be considered uncertain as they

TABLE VI. Patients who died of myocardial infarction

	n	$\bar{x}$	SD	$\varepsilon_{\bar{x}}$
Age, years	24	72.5	8.8	1.8
Height, cm	22	177.5	6.0	1.3
Body weight, kg	22	71.3	11.6	2.5
Systolic B.P., mm Hg	23	153	38	8
Diastolic B.P., mm Hg	23	92	17	3
Time in hospital, days	24	2.6	2.9	0.6

were measured under threatening cardiogenic shock. The mean time in hospital was 2.6 days (range 0—12). Within two days of admission 16 and within three days 20 (83 %) had died. Postmortems of these 20 patients showed that four had heart rupture, i.e. 16.8 % of all 24 deaths. Of the remaining 20 post mortem showed death to have been caused by the infarction as such in 19 cases. An 88 year old man was considered to have died of a complicating pneumonia. Anterior wall infarction occurred in ten cases (41.6 %), posterior wall infarction in seven (29.1 %), septal infarction in two and apical in one of the 24 myocardial infarction patients.

Of the six patients who died before the follow up three had died suddenly in the home, probably owing to reinfarction. One died of lung embolism, one of uraemia and one of reinfarction treated in the hospital in January 1966.

Discussion

During 1965 the Danderyd Hospital treated 110 patients on a diagnosis of myocardial infarction. As the population of the hospital reception area is rather more than 200,000, the incidence of myocardial infarction is about five per 10,000. This is a distinctly lower figure than found by Biörck et al. in their study from Malmö (3), where the incidence was 15—20 per 10,000 in a reception area of roughly equal size. This difference is undoubtedly due to the recent addition of many young families in the north Stockholm suburbs.

The mean cholesterol and triglyceride levels for the entire material of 73 cases was clearly elevated compared with earlier reported normal materials (7, 8). For the men of 50 years of age (table II) the mean cholesterol level was clearly higher than for healthy 50 year old men in Gothenburg (27). Compared also with the Värmland health survey (17), in which only 2—3 % had elevated cholesterol levels, the mean level in the present study was high. Comparisons with normal materials thus show that the infarction patients generally have higher serum lipids than is usual, which agrees well with the results of earlier studies (1, 2, 6, 8, 12).

The division of the material into age groups (table II) shows there to have been 25 patients between 66 and 79

years and none below 40 In this respect the material differed from that of Carlson (8) A comparison with a normal material earlier studied by Carlson (7) shows that the two youngest age groups in the present material had especially high cholesterol levels This is in close accordance with the results of Biorck et al (2, 6) Compared with the same normal material (7) the triglyceride levels were high especially for the youngest and oldest age groups A study of the distribution of high and low cholesterol and triglycerides (tables III and IV) however, showed — though not significantly—that the highest values in both cases appeared to be in the youngest patients, the lowest in the oldest patients The reason may be that many of the patients with high cholesterol and triglycerides die of myocardial infarction or other vascular disease at a younger age

In the present infarction material elevated serum lipid values were found in 43 (59 %) of the 73 patients Thus the serum lipid values were normal in no less than 41 % of the cases (table III) This is a considerably higher figure than reported earlier (1) but need not mean that these patients have a normal lipid metabolism It is possible that disorders of the lipid metabolism are not always revealed in the form of elevated serum lipids An association with changed carbohydrate metabolism is also conceivable This has earlier been intimated by Biorck (5) and later described by Wahlberg (28)

A distinct enlargement of the heart volume was found in 37 % of the cases A very slightly higher percentage was reported in a recent study (29), in which it was also found that the survival period for those with enlarged heart after myocardial infarction was shorter than for others A similar prognosis may therefore be expected for the patients with enlarged heart in the present study

No significant difference was found between the 49 who stated that they avoided fat foods and the other 24 in respect of weight, skinfold thickness cholesterol or triglycerides (table V) This might be due to the fact that those who reported that they avoided fat foods did not actually do so to the full extent Another possible explanation is that those who did not particularly avoid fat foods nevertheless did not eat such foods in any large quantities The high values of serum lipids recorded for most patients (tables III and IV) might also have been caused by an endogenous disorder of the fat metabolism

Mental stress was reported by 57 5 % of the patients to have been an active cause of their illness From earlier investigations (10 11) it is known that the free fatty acid metabolism is affected by mental stress probably via the autonomic nervous system It is likely that the lipid metabolism is affected as well Mental stress might therefore induce a disorder of the fat metabolism which could indirectly give rise to a myocardial infarction There is however no definite evidence that this is so

Of the 110 hospitalized infarction patients 27 (24 5 %) died in hospital three of whom with diabetes mellitus Of the remaining 24 deaths 83 % occurred within three days which is in close accordance with earlier results (19

26). Heart rupture occurred in 16.8 % of these cases, a rather higher figure than reported by Biorck et al. in 1966. The number of deaths in the present study was fairly low compared with the figures of Biorck et al. (4). But as pointed out by Helander (14), the severity of the infarction should be stated in such comparisons. As far as can be judged from the maximal rise of GOT, 35 % of the patients in the present study having values below 100 Karmen units, the number of mild infarctions appears to have been fairly large. This might explain why the hospital mortality was fairly low. The time in hospital (22.6 days) varied greatly (range 7—60 days), which must be considered to be due to very varying size of infarctions and difference in clinical course.

Summary

All patients treated during 1965 at the Danderyd Hospital on a diagnosis of myocardial infarction have been followed up about one year after discharge. Of the original number of 110, 27 (24.5 %) died in hospital. Two of the remaining 83 had diabetes mellitus and have been excluded. Six died before the follow up examination. Two patients could not be induced to come to the examination. Of the 81 discharged patients, accordingly, 73 (90.1 %) were followed up.

Of the 27 who died, 3 had diabetes mellitus. These have been excluded. Of the remaining 24, 20 (83 %) died within three days. In four cases (16.8 %) the cause of death was heart rupture.

The mean age of the 73 followed up patients was 60 years. In body build they were shorter, more heavily built and rather fatter than a normal population. In this respect they resembled mostly weight lifters and wrestlers earlier described by Hellstrom (15).

Eighteen cases (24.7 %) had high levels both of cholesterol and triglycerides, ten (13.7 %) high cholesterol with normal triglycerides, and 15 (20.6 %) high triglycerides with normal cholesterol. Serum lipids were thus elevated in 59 % of the cases. The normal levels of cholesterol and triglycerides in the remaining 41 % were not considered to exclude the possibility of a disturbed fat and carbohydrate metabolism. The highest values of cholesterol and/or triglycerides seemed to exist in the youngest patients. No significant correlation was found between, on the one hand, cholesterol and triglycerides and, on the other, age, weight, femoral condyle width and skinfold thickness. Nor was there any significant difference in weight, skinfold thickness, cholesterol and triglycerides between the 49 patients who stated that they particularly avoided fat food and the other 24 who did not keep a dietary regimen at all.

The heart volume, determined in standing position per sq. m body area, was distinctly enlarged in 37 % of the cases. The systolic blood pressure was in 42.5 % of the cases above 150 mm Hg, in 10.9 % above 200 mm Hg and the diastolic above 100 mm in 34.2 %.

In 35 % of the cases the GOT level did not rise above 100 Karmen units. Rises to between 100—200 were recorded in 44 % and above 200 in 21 %. The relatively large number of mild cases

was cons dered to be the reason for the fairly small number of deaths (24 5 %) in hospital

The mean time in hospital of the infarction patients was 22 6 days but with large variations (range 7—60)

Mental stress as an active cause of the illness was reported by 57.5 % of the patients Of the 73 infarction patients 11 (15 1 %) had earlier had an infarction all prior to 1960 An equal number had had an X ray verified peptic ulcer Eleven of the patients finally had had a parent with myocardial infarction and six a parent with peptic ulcer

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Glucose Metabolism in Parathyroid Disease

By

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The observation of an enhancing effect of parathyroid hormone on the tubular reabsorptive capacity for glucose (1) has made it desirable to know whether parathyroid hormone exerts any influence on other parameters of glucose metabolism in vivo A review of the literature reveals vague and in many respects conflicting results of investigations on this subject No conclusion can be drawn as to whether or not there is an endocrine connection between the parathyroid glands and the glucose metabolism

The present study was carried out to evaluate some data of interest concerning the glucose metabolism in clinical hyper and hypoparathyroidism

Material and methods

Ten patients with parathyroid diseases were elected for the study Five had postoperative hypoparathyroidism and five primary hyperparathyroidism In all patients an intravenous glucose tolerance test was performed In the hypoparathyroid patients the glucose test was repeated after i m administration of 200 I U of parathyroid extract (Parathormone® 100 U S P parathyroid units/ml, Eli Lilly & Co) twice a day for 2—3 days

In the hyperparathyroid patients the glucose test was repeated four to seven days after successful removal of parathyroid adenomas In three of the hypoparathyroid patients the glucose test was immediately followed by an insulin responsiveness test before and after the administration of parathyroid extract

All tests were done at 9 a m after an overnight fast A blood sample was obtained through a needle in an antecubital vein following which 50 ml of 50 % solution of glucose in water were injected rapidly through the same needle Subsequently an indwelling needle was placed in the opposite antecubital vein and blood specimens were obtained at intervals of ten min In two of the hypoparathyroid patients five blood samples were drawn at 1—2 min intervals within ten min after injection of glucose In three of the hypoparathyroid patients, 0 1 units of insulin/kg body weight were injected intravenously 60 min after the glucose injection, and the collection of blood samples was continued at intervals of five min for the following 30 min The blood samples were collected in tubes containing dry sodium fluoride and centrifuged immediately after the collection Blood glucose concentration was determined on the test day in duplicate by a modified glucose oxidase method (1

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Data analysis

In each test the responses to glucose and insulin administration were analyzed by plotting the logarithm of the blood glucose concentration against time. A linear relationship was obtained, demonstrating that the blood glucose concentration decreased at an exponential rate. The slope of the linear part of the curve provides indices of the response to glucose (or insulin) administration K_G or K_I = the per cent fall in blood glucose concentration per minute. These indices may be derived from the formula

$$K_G \text{ or } K_I = \frac{\log_e 2}{t^{1/2}} \times 100$$

where $t^{1/2}$ is the glucose half time in minutes.

Another linear relationship was obtained by plotting the logarithm of the blood glucose concentration at 1—2 minute intervals within ten min. after the glucose injection as measured in two of the patients. The slope of this linear curve provides indices of the immediate rate of distribution of intravenously administrated glucose. K_A = per cent fall in blood glucose concentration per minute.

An estimate of the volume of distribution of glucose (VD_G) was obtained by applying the formula

$$VD_G = \frac{N}{A_G - FBS} \text{ liters}$$

where N is the total amount of glucose injected (in mg), A_G is the teoretical glucose concentration (mg/100 ml) at zero time obtained by extrapolation of the linear part of the glucose curve to zero time, and FBS is the fasting blood glucose concentration.

During the linear part of the curve the mean cellular glucose uptake/min/kg body weight (MCU_G) may be derived from the expression

$$MCU_G = \frac{VD_G \times A_G (1-e^{-\frac{K_G \times t}{100}}) \times 1000}{w \times t \times 100}$$

where w is body weight in kg and t is time in minutes. (The principles of data analysis in this study have been copied from Sagild et al. (2).)

Results

The results are stated in table I. No significant changes of the parameters of glucose metabolism used in this study were observed.

Discussion

We do not know the duration of the hypoparathyroid state following extirpation of a solitary adenoma because probably, the decreased serum calcium concentration immediately stimulates the remaining parathyroid glands to increase their hormone secretion.

The patients might therefore be in a state of secondary hyperparathyroidism as early as the 4th to 7th post operative day. A more distinct separation between hypo- and hyperparathyroidism exists in the hypoparathyroid patients receiving parathyroid extract as measured by significantly increased serum calcium concentrations. The data of glucose metabolism presented here, however, are quite uniform for all four groups of patients (table I: a, b, c, and d).

From the data presented it is concluded that parathyroid hormone does not exert any influence of significance on the glucose metabolism in vivo. However, a slight tendency of the parathyroid hormone to decrease K_G (hence MCU_G) might be expected because of the enhancing effect of the hormone on the tubular reabsorption of glucose (TmG). In patient no. 1 TmG increased from 104 to 166 mg/min. and in patient no. 2 from 72 to 116 mg/min. after administration of parathyroid extract. In patient no. 7 TmG decreased from 217 to 184

TABLE I Determination of FBS (mg/100 ml) K_G K_I K_a VD_G% (VD_G in % of body weight) and MCU_G (mg/kg body weight/min) in ten patients with clinical hypo- (a and c) and hyperparathyroidism (b and d)

Five patients with hypoparathyroidism before (a) and after (b) administration of parathyroid extract

Pat no	1		2		3		4		5		Mean	
	a	b	a	b	a	b	a	b	a	b	a	b
FBS	77	64	84	88	72	88	72	80	79	87	76.8	81.4
K_G	1.31	1.06	1.72	1.26	0.92	1.14	1.74	1.91	1.27	1.40	1.39	1.35
K_I	3.88	3.26	8.83	8.63	4.72	4.85	—	—	—	—	5.81	5.58
K_a	—	—	—	—	—	—	4.92	5.15	4.02	3.99	4.47	4.57
VD_G%	15.4	15.7	25.7	24.3	23.1	18.4	14.5	10.8	20.2	19.8	19.8	17.8
MCU_G	3.75	3.07	7.15	5.62	3.53	4.37	5.52	5.58	4.88	5.39	4.97	4.81

Five patients with parathyroid adenoma before (d) and after (c) operation

Pat no	6		7		8		9		10		Mean	
	c	d	c	d	c	d	c	d	c	d	c	d
FBS	82	78	81	77	76	74	106	106	74	81	83.8	83.2
K_G	1.74	1.70	2.11	1.99	1.12	1.35	0.60	0.64	1.04	1.05	1.32	1.35
VD_G%	15.6	16.5	17.5	18.8	19.3	16.3	19.2	16.8	19.0	21.4	18.1	18.0
MCU_G	7.42	7.28	8.16	7.90	4.23	4.41	2.62	2.66	4.03	3.35	5.29	5.12

mg/min after removal of a parathyroid adenoma. Provided that

1) parathyroid extract does not change the splay on the curve that shows the relation of renal load of glucose to tubular reabsorption of glucose,

and that

2) the altered glucose elimination from VD_G caused by parathyroid extract alone is due to changes of urinary excretion of glucose

it is possible to determine how much the enhancing effect of parathyroid hormone on tubular reabsorption of glucose modifies the elimination of glucose from VD_G when the values of blood glucose, tubular reabsorption of glucose and glomerular filtration rate are known. The difference of excreted glucose per minute in per cent indicates the change of the K_G value due to parathyroid hormone. The calculated decrease of K_G in patients no 1, 2 and 7 was 0.098 (0.093—0.100—

0.102), i.e. the increased tubular reabsorption of glucose counts for a 9.8 % decrease of the per cent fall in blood glucose per minute. A decrease of K_G of 10 % will cause a decrease of MCU_G of 6—8 %. These changes, however, are too insignificant to be detected by the technique used in this study.

Summary

Determinations of some data of glucose metabolism were carried out in five patients with hypoparathyroidism before and after administration of parathyroid extract and in five patients with primary hyperparathyroidism before and after removal of parathyroid adenomas. No significant changes were observed.

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TABLE I Clinical and laboratory data in 28 adult nephrotic patients

Pat no	Sex	Age (yrs)	Diagnosis[1]	B P (mm Hg)	Hb (g/100 ml)	ESR (mm hr)	Serum albumin (g/100 ml)
1	♂	39	Rheum arthrit (sanocrysin poison) N.S	110/75	16 7	25	0 83
2	♂	37	Lead poison N S	110/90	15 2	3	1 09
3	♀	27	N S in pregnancy	125 85	10 3	91	1 08
4	♀	21	N S	130/85	11 0	131	0 28
5	♀	19	N S	120/85	14 3	91	0 97
6	♂	56	N S	150/100	16 9	113	1 01
7	♀	60	N.S	175/100	12 6	54	1 51
8	♀	58	N S	120/90	14 3	94	0 64
9	♀	41	N S	110/80	13 3	51	0 49
10	*♀*	*69*	*N S*	*150/75*	*12 6*	*73*	*0 83*
11	♂	24	N S	120/75	13 8	61	0 88
12	♂	60	N.S	145 90	10 2	45	0 99
13	♂	14	N.S	120/95	13 5	106	1
14	♂	17	N S	130/95	15 3	35	
15	♂	44	N S	110/75	11 0	103	
16	♀	52	N S	160/85	11 2	84	
17	♂	60	Diab mell N S	140/90	11 5		
18	♂	51	N S	150/85	13 0		
19	♀	46	N S	120/80	13 8		
20	♂	38	N S	140/90	13 1		
21	♂	42	N S	140/90	13 3		
22	♂	57	Renal vein thrombosis N.S	165/95	14 6		
23	♂	24	N S	120/70	15 3		
24	♂	41	N S	150/90	13 0		
25	♂	43	N S	140/75	17 6		
26		55	N S	150/90	11 7		
27		68	Dermatitis herpetiform N S	140 80	13 0		
28	♂	14	N S	140/100	12 8		
Normal values (mean ± 2 SD					♀ 13 3—16 3 ♂ 14 8—18 5		

[1] N.S = nephrotic syndrome

with a selective glomerular permeability of plasma proteins with relatively low molecular weights (especially albumin) respond well in contrast to nephrotics with a non selective glomerular permeability (5, 9, 10, 19, 22 40)

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Serum $\alpha_1$glob (g/100 ml)	Serum βglob (g/100 ml)	Serum γglob (g/100 ml)	Serum cholesterol (mg/100 ml)	Serum creatinine (mg/100 ml)	Proteinuria (g/24 hrs)
0 96	1 05	0 63	699	1 0	12 0
0 69	0 82	0 72	606	1 4	8 0
0 98	0 66	0 75	510	0 9	14 8
1 75	0 50	0 79	655	0 8	8 0
1 28	0 49	0 54	990	0 7	20 0
0 85	1 26	0 60	584	2 3	8 0
1 49	0 80	0 58	834	1 6	7 5
1 39	0 96	1 00	612	1 5	16 5
0 85	0 86	0 81	695	0 8	19 4
1 08	0 91	0 64	787	1 2	7 7
1 43	0 76	0 59	650	0 8	6 7
1 20	0 55	0 65	368	2 0	9 8
1 48	0 84	0 57	549	1 1	5 0
1 10	0 47	0 60	467	0 9	12 8
0 85	0 75	0 54	683	1 2	6 3
0 94	0 61	0 65	380	2 7	14 6
1 28	0 81	0 80	480	1 1	3 5
1 04	0 75	0 50	565	1 1	14 0
0 82	0 67	0 39	730	0 7	8 4
1 02	0 87	0 43	372	0 7	3 5
0 67	0 90	0 59	788	1 7	9 0
0 92	0 70	0 42	433	0 9	14 8
1 10	0 71	0 40	916	0 7	8 8
0 96	0 95	1 41	378	1 5	11 8
0 96	0 91	0 74	561	1 1	11 0
0 71	0 69	0 96	565	0 7	5 7
0 51	0 81	0 95	512	1 2	3 5
0 68	0 72	0 55	412	0 8	7 2
0 31—0 59	0 47—0 79	0 62—1 10	150—300	<1 3	< 0 150

plasma proteins with lower molecular weights but in some cases of nephrosis no correlation is present Therefore determination of glomerular clearance of different plasma proteins is an important adjuvant to kidney biopsy in evaluating nephrotic patients for steroid treatment

It is the purpose of the present paper to report on the initial response to steroid treatment in 28 adult nephrotics especially in relation to kidney biopsy and plasma protein turnover studies

TABLE I Clinical and laboratory data in 28 adult nephrotic patients

Pat no	Sex	Age (yrs)	Diagnosis[1]	B P (mm Hg)	Hb (g/100 ml)	ESR (mm hr)	Serum albumin (g/100 ml)
1	♂	39	Rheum arthrit (sanocrysin poison) N S	110/75	16 7	25	0 83
2	♂	37	Lead poison N S	110 90	15 2	3	1 09
3	♀	27	N S in pregnancy	125 85	10 3	91	1 08
4	♀	21	N S	130 85	11 0	131	0 28
5	♀	19	N S	120 85	14 3	91	0 97
6	♂	56	N S	150/100	16 9	113	1 01
7	♀	60	N S	175/100	12 6	54	1 51
8	♀	58	N S	120/90	14 3	94	0 64
9	♀	41	N S	110 80	13 3	51	0 49
10	♀	69	N S	150/75	12 6	73	0 83
11	♂	24	N S	120/75	13 8	61	0 88
12	♂	60	N S	145 90	10 2	45	0 99
13	♂	14	N S	120 95	13 5	106	1 01
14	♂	17	N S	130/95	15 3	35	1 06
15	♂	44	N S	110/75	11 0	103	1 20
16	♀	52	N S	160 85	11 2	84	1 29
17	♂	60	Diab mell N S	140 90	11 5	126	1 43
18	♂	51	N S	150 85	13 0	66	1 45
19	♂	46	N S	120 80	13 8	66	1 62
20	♂	38	N S	140 90	13 1	45	1 66
21	♂	42	N S	140 90	13 3	45	1 72
22	♂	57	Renal vein thrombosis N S	165 95	14 6	48	1 80
23	♂	24	N S	120 70	15 3	41	1 85
24	♂	41	N S	150 90	13 0	110	1 95
25	♂	43	N S	140 75	17 6	23	2 26
26		55	N S	150 90	11 7	67	2 63
27		68	Dermatitis herpetiform N S	140 80	13 0	30	2 90
28	♂	14	N S	140 100	12 8	42	3 00
Normal values (mean ± 2 SD)					♀ 13 3—16 3 ♂ 14 8—18 5	♀ 2—10 ♂ 2— 6	4 44—5 86

[1] N S = nephrotic syndrome

with a selective glomerular permeability of plasma proteins with relatively low molecular weights (especially albumin) respond well in contrast to nephrotics with a non selective glomerular permeability (5 9, 10, 19, 22 40)

Generally the microscopic examination and glomerular clearance pattern of plasma proteins are correlated in such a manner that minor alterations in microscopic examination correspond to a selective glomerular permeability of

Type C response indicates reduction of proteinuria and some diuresis, but no alteration in serum proteins and cholesterol

In type D response the treatment was without any effect

Results of investigations

Kidney biopsy

The material consists of 22 kidney biopsies from 21 patients

The histological findings are listed in table II The various changes found in the glomeruli, tubuli, interstitial tissue and vessels have been listed in a semi quantitative way as follows

—	no changes
(+)	very slight or doubtful changes
+	slight, but definite changes
++	pronounced changes
+++	severe damage

These changes have been grouped into four terms

1 Minor alterations, which means that the histological picture is either normal, as seen by the light microscope or the changes are very slight

2 Membranous glomerulonephritis (+), +, ++, +++

3 Proliferative glomerulonephritis (+) +, ++, +++

4 Membranous proliferative changes (+), +, ++, +++

This nomenclature is the same as that used by Brewer (6) and has been found suitable to the present material

Plasma protein turnover studies

Albumin

Fractional disappearance rate (FDR) was elevated in all 15 examined cases (18—89 %) Fractional catabolic rate (FCR) was increased in nine patients The synthetic rate was increased in four cases and normal in the remaining 11

IgG

FDR was elevated in all six cases (14—40 %)

FCR was increased in four (11—18 %)

The synthetic rate was normal in one case, decreased in two, and increased in three The results are summarized in table III

Selectivity index varied from 0 30 to 0 64 (table IV)

Results of treatment

A In the whole material

The initial response to prednisone in the 28 patients was

Type A	11	(39 %)
Type B	6	(22 %)
Type C	4	(14 %)
Type D	7	(25 %)

The average value of various laboratory tests in the four response groups is given in table V Fig 1 illustrates typical response of type A, B and C, and shows that in type A and B response diuresis appeared and proteinuria disappeared or diminished within about 10 (8—16) days after initiation of the therapy The reaction usually set in rather abruptly

Only these reactions were considered due to steroid treatment In pat ents in

TABLE II Histological findings in kidney biopsies in 21 adult nephrotic patients

Pat no	No of glomeruli	Hyper cellularity	Thickening of basement membrane	Synechias	Crescents	Fibrosis
8	Autopsy	– focal	—	—	—	—
9	13	(+)	—	+	—	—
10	15	(+) focal	—	—	—	—
11	6	— ?	—	—	—	—
12	10	+ focal	++	+	—	+
13	30	—	—	—	—	—
14	10	+ focal	—	(+)	—	—
15	9	+ focal	—	+	—	—
16	Surgical biopsy	++	—	++	—	—
17	10	—	+ focal	+	—	—
18	6	++	—	—	—	+
19	10	—	—	—	—	—
20	10	—	+	—	—	—
21	20	+ focal	—	+	—	—
22	10	(+)	— ?	—	—	—
23	10	—	?	—	—	—
24	1	+	+	—	—	—
25	30	—	+	(+)	—	—
	30	—	(+)	—	—	—
26	20	—	(+)	—	—	—
27	10	—	(+)	—	—	—
28	10	+ + focal	(+)	+	—	—

whom diuresis increased and proteinuria disappeared or diminished after lengthy treatment (weeks or months) with small doses of steroid the remission was not attributed to steroids.

B In relation to kidney histology

Table VI shows the histological diagnosis compared with the results of the treatment

It is seen that the patients with minor alterations in the kidney biopsy with one exception (no 22) showed an excellent response to the treatment Furthermore it is seen that also in the group of membranous and proliferative glomerulonephritis a good response was seen if the changes were slight

C In relation to plasma protein metabolism

In the 15 cases investigated with radioiodinated albumin four responses were of type A five of type B, and six of type D (table VII) In the four patients with type A response the average FDR was 43 % (38–49) in the groups with B and D response 47 % (25—89) and

Tubuli cylinders	Tubuli atrophy	Vessels	Diagnosis
++	—	—	Proliferative +
+	—	—	Minor alterations
(+)	—	—	Proliferative (+)
—	—	—	Minor alterations
+	+	—	Membranous-proliferative ++
+	—	—	Minor alterations
—	—	—	Proliferative +
(+)	—	—	Proliferative +
(+)	—	Cellular thickening	Proliferative ++
—	—	Cellular thickening	Membranous +
(+)	—	—	Proliferative ++
(+)	—	—	Minor alterations
(+)	—	—	Focal membranous +
—	—	—	Proliferative +
+	—	—	Minor alterations
—	—	—	Minor alterations
—	—	—	Membranous-proliferative +
(+)	—	—	Membranous +
—	—	—	Membranous (+)
—	—	—	Minor alterations
+	—	—	Membranous (+)
—	—	—	Proliferative ++

24 % (18—36) respectively. The average FCR in the three groups was 22 % (19—24), 18 % (9—33) and 11 % (9—14) respectively, and FDR—PB 21 %, 28 % and 13 %.

The synthetic rate of albumin in the four patients with A response was on an average 183 mg/kg day (148—210). In the groups with B and D response it was 217 mg/kg day (182—272) and 201 mg/kg day (129—286).

The result of steroid treatment in the six patients examined with IgG was as follows:

Type A response 1 case
Type B response 2 cases
Type D response 3 cases

FCR was 15 % in the one patient with A response and on an average 15 % and 9 % in the other two groups.

In the one patient with A response the synthetic rate of IgG amounted to 30 mg/kg/day. In the groups with B and D response the average value was 54 mg/kg/day (26—81) and 69 mg/kg/day (19—111), respectively.

The selectivity index in the patient with *A response* was 0.25, *in the two*

TABLE III Albumin and IgG metabolism in 15 adult nephrotic patients

Pat no	Albumin				IgG			
	FDR	FCR	FDR PB	S	FDR	FCR	FDR PB	S
8	89	33	56	182	40	18	22	81
10	49	22	27	187	22	15	7	30
11	42	24	18	148				
12	18	10	8	129	14	9	5	111
14	50	23	27	272				
15	26	13	13	182	15	11	4	26
16	43	9	34	261				
17	24	14	10	134				
18	26	9	17	184	14	7	7	19
19	42	19	23	188				
22	36	14	22	205				
23	38	22	16	210				
24	20	9	11	270	16	12	4	78
26	25	14	11	190				
28	22	11	11	286				
Normal range	7—11	7—11	0	136—241	4—9	4—9	0	[1]29—43

[1] Andersen (2)

FDR = fractional disappearance rate per cent of intravascular mass disappearing per day from plasma by way of catabolism and external loss
FCR = fractional catabolic rate per cent of intravascular mass degraded per day
FDR PB = protein bound fractional disappearance rate per cent of intravascular mass excreted in urine per day
S synthetic rate (mg/kg/day)

TABLE IV Index of selectivity in six adult nephrotic patients

Pat no	Renal clearance (ml/day)		Index of selectivity
	Albumin (a)	IgG (b)	b/a
8	1 600	594	0 37
10	540	133	0 25
12	392	250	0 64
15	480	144	0 30
18	595	238	0 40
24	450	148	0 33

cases with B response 0 30 and 0 37 Finally the selectivity index in the three patients with D response was on an average 0 46 (0 33—0 64)

Discussion

The five patients with a *secondary nephrotic syndrome* require special comment

In patient no 1 who developed a nephrotic syndrome after gold therapy (2 3 g sodium aurothiosulfate) for rheumatoid arthritis steroid treatment re-

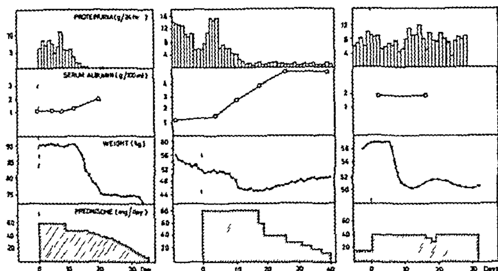


Fig. 1. From left to right typical response of type A, B and C obtained on prednisone treatment of the nephrotic syndrome in adults.

TABLE V. Mean values of various parameters in relation to initial steroid response in 28 adult nephrotic patients

Type of response	Age (yrs)	Serum albumin (g/100 ml)	Serum α globulin (g/100 ml)	Serum cholesterol (mg/100 ml)	Serum creatinine (mg/100 ml)	Proteinuria (g/24 hrs)
A (n = 11)	37	1.1	1.1	690	1.1	9.8
B (n = 6)	42	1.3	1.0	540	1.3	11.8
C (n = 4)	53	2.1	0.9	680	1.6	7.8
D (n = 7)	43	1.6	1.1	470	1.2	9.9

sulted in a type A response. This has also been observed by other investigators (7, 18, 26, 38).

Lead poisoning is known to cause proteinuria and glomerulonephritis (36), but development of a *real nephrotic* syndrome as in patient no. 2 in this study has not earlier been published. The result of steroid treatment was a complete remission.

Nephrotic syndrome and pregnancy is a rare constellation (15, 24, 32, 35, 41) and it is always difficult to ascertain a causal relationship, especially when no histological examination of kidney tissue is performed. The patient (no. 3) in

TABLE VI Relation between histological diagnosis and response to steroid therapy in 21 adult nephrotic patients

Minor alterations	A A A A A B D[1]
Membranous	
glomerulonephritis (+)	C
+	A C D[2]
Proliferative	
glomerulonephritis (+)	A
+	B B B C
++	B D D
Membranous proliferative	
glomerulonephritis +	D
++	D

[1] Patient no 22 with renal vein thrombosis
[2] Patient no 17 with diabetes mellitus

this study developed a typical nephrotic syndrome in the seventh month of her first pregnancy One month after the delivery she was treated with prednisone The result of the treatment was a type B response

Patient no 17 had diabetes mellitus and a nephrotic syndrome As the histological picture of the kidney biopsy showed no specific diabetic lesions in the glomeruli, but only thickening of the wall in the small arteries, the patient was treated with steroids but without effect

Patient no 22 showed minor alterations in the kidney biopsy, but did not show any response at all to the steroid treatment It was subsequently found by autopsy that this patient suffered from bilateral renal vein thrombosis The histological picture of the kidney biopsy was not incompatible with this diagnosis When the latter two patients are omitted from the material, the close correlation between the histological changes and the result of treatment discussed below becomes even more apparent

In every discussion of steroid treatment of the *primary nephrotic syndrome* the question arises whether the beneficial

TABLE VII Initial response to steroid treatment in relation to albumin metabolism in 15 adult nephrotic patients

Type of response	FDR mean/range	FCR mean/range	FDR PB mean/range	S mean/range
A	43	22	21	183
(n = 4)	(38—49)	(19—24)	(16—27)	(148—210)
B	47	18	28	217
(n = 5)	(25—89)	(9—33)	(11—56)	(182—272)
D	24	11	13	201
(n = 6)	(8—36)	(9—14)	(8—22)	(129—286)
Normal range	7—11	7—11	0	136—241
	%/day	%/day		mg/kg/day

FDR = fractional disappearance rate per cent of intravascular mass disappearing per day from the plasma by way of catabolism and external loss
FCR = fractional catabolic rate per cent of intravascular mass degraded per day
FDR PB = protein bound fractional disappearance rate per cent of intravascular mass excreted in urine per day
S = synthetic rate

effect observed is caused by the steroids or by a spontaneous remission, which is likely to occur in this disease

Nesson et al (29) found a remission in 27 of 143 cases of untreated idiopathic nephrotic syndrome in adults gathered from the literature In other words this means that a beneficial effect can only be ascribed to steroids if the value for type A and B response exceeds about 20 %

Adams et al (1) found type A and B response in 43 % of 157 adult nephrotics gathered from the literature This figure is in agreement with that obtained by later investigators (8, 16 29 31 33, 39), who found type A and B response in from 29 to 51 per cent In the present material type A and B response was obtained in 14 of 23 adult nephrotics with verified or presumed idiopathic nephrotic syndrome i e in 65 %

These results indicate that steroids are of great value in the treatment of idiopathic nephrotic syndrome in adults as regards initial response, and that the beneficial effect is not caused by a coincidental spontaneous remission

Another point in favour of a causal relationship between the steroid treatment and the remission obtained was the relatively short period (8—16 days) after initiation of the therapy within which diuresis appeared and proteinuria disappeared or diminished in patients with type A and B response in this study

In agreement with earlier investigators the present material demonstrates that the most favourable effect of steroid treatment is obtained in cases with no or few abnormalities in the light microscopic picture of kidney tissue In cases of slight, but definite lesions some investigators have observed the best result of steroid treatment in cases with proliferative glomerular lesions (5, 8, 29, 30), and others in cases of membranous glomerulonephritis (12 16 33, 35, 37, 39) Judged from the very few patients in the present material, it seems that patients with proliferative glomerulonephritis might show a somewhat better response than those with membranous glomerulonephritis

Proteinuria is an important factor in the pathogenesis of the hypoproteinaemia in the nephrotic syndrome Besides this a hypercatabolism has been found in several cases (for references see Andersen et al (3) Jensen et al (21)) The cause of this hypercatabolism is still unknown but a degradation of plasma proteins within the nephrotic kidney is as yet the most plausible explanation (21)

In the present study albumin turnover studies were performed in 15 patients In four cases with type A and five cases with type B response to steroid treatment the average fractional catabolic rate was 22 (19—24) and 18 (9—33) % per day, respectively, whereas in six patients with type D response the figure was 11 (9—14) % per day (table VII) This finding agrees with the results seen in table V The lowest serum albumin concentration and highest proteinuria were found in cases of type A and B response

Although the present material is small, the results seem to indicate that the most favourable results of steroid treatment are obtained in cases with the highest fractional catabolic rate of al-

bumin and probably also of other plasma proteins

Andersen et al (3) found an increased synthetic rate of IgG in eight of 13 cases of idiopathic nephrotic syndrome in adults and took this observation as evidence of some immunomechanism operative in these cases of nephrosis In other diseases with an immunological pathogenesis steroids are known to be of great value In the present series IgG turnover studies were performed only in six patients and no correlation was found between the synthetic rate of IgG and the response to steroid treatment

Summary

Twenty eight adult nephrotics were treated with steroids Twenty three had an idiopathic nephrotic syndrome In the remaining five cases the syndrome was caused by poisoning (two cases) pregnancy (one), diabetes mellitus (one), and renal vein thrombosis (one)

Kidney biopsy was performed in 21 cases, and turnover studies with radioiodinated albumin and IgG in 15 and six cases respectively

The results of steroid treatment are discussed in relation to the light microscopic picture of kidney tissue and to turnover studies with labelled plasma proteins

The best result of steroid treatment in the idiopathic nephrotic syndrome was obtained in patients in whom 1) the histological picture of kidney tissue showed minor alterations or slight proliferative lesions in the glomeruli 2) the fractional catabolic rate was increased, 3) the glomerular permeability was selective to albumin

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Effects of Acetylcholine and Isoprenalin on Pulmonary Circulation in Bronchial Asthma

By

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In a previous study (10) we examined the effect of acetylcholine infusion on the pulmonary circulation in patients with bronchial asthma who were examined during a symptom free period The results could be taken to indicate that there was significant pulmonary vasoconstriction in discrete lung regions, in spite of absence of pulmonary hypertension at rest During infusion of acetylcholine in the pulmonary artery a mean reduction of the arterial oxygen saturation from 95 2 to 88 7 % was recorded The observed signs of reversibility of the vascular changes agree with earlier observed signs of reversibility of cardio pulmonary functions in patients with bronchial asthma

The purpose of the present survey is to study the extent to which the reduction of the arterial oxygen saturation brought about by infusion of acetylcholine is influenced by bronchodilating drugs The definition of bronchial asthma followed the recommendations published by the American Thoracic Society (1) This means that the present study does not include patients with chronic bronchitis

Material

The present investigation was performed on ten patients The degree of severity of the bronchial asthma was assessed by a method earlier described (3) for the whole period from the onset of the disease as well as for the five year period immediately preceding the investigation The patients were classified into three severity groups A and a = moderately severe asthma B and b = severe asthma C and c = very severe asthma capital letters refer to the assessed severity of the disease since its onset and small letters to the five years immdiately preceding the present investigation (table I) According to this evaluation and with regard to the whole period since the onset, six patients had had very severe asthma three severe and one moderately severe asthma With regard only to the five years immediately preceding the investigation four patients had

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TABLE I Individual data and mean values showing the assessed degree of asthma severity, ventilation capacity and lung volumes

Pat	Sex	Age	Groups according to degree of severity since onset of the asthma	Groups according to degree of severity of the asthma for last five years	Age at onset	Duration of disease (yrs)	BSA (m^2)	VC (% of pred)	
								b i	a i
A S	♂	56	B	c	51	5	1 94	67 3	70 0
R O	♂	54	B	c	49	5	1 70	88 4	90 7
H H	♂	49	A	a	20	29	2 00	115 3	113 5
K A	♂	45	C	c	35	10	2 02	70 6	72 6
B E	♀	54	C	c	22	33	1 46	96 4	96 4
I N	♀	44	C	a	6	38	1 56	100 0	100 0
M S	♀	43	C	b	19	33	1 81	93 9	100 0
B F	♀	34	C	b	14	20	1 60	95 2	95 2
L R	♀	33	C	b	17	16	1 52	94 9	94 9
B F	♀	24	B	b	19	5	1 70	100 0	100 0
Mean		43 6			25 2	19 4	1 73	92 2	93 3
Highest		56			51	33	2 02	115 3	113 5
Lowest		24			6	5	1 46	67 3	70 0

BSA body surface area b i = before isoprenalin a i = after isoprenalin

had a v ry severe asthma four severe and two moderately severe asthma The mean age at onset was 25 years and the mean duration 3 of the disease 19 years

All tests of respiratory and circulatory function were made during an asthma free interval which means that during the period of the tests and for at least two days previously the patient was either entirely free from symptoms or in what he regarded as his most symptom free state for the last twelve months On auscultation of the lungs during normal breathing there should either be no rhonchi heard or the above mentioned criterion for the most optimal state should apply

Methods

The methods for analyses of respiratory and circulatory function have been described previously (3) At the right heart catheterisation circulation studies comprising acetylcholine infusion in the pulmonary artery were performed before and after isoprenalin inhalation and also during a work test with the patient supine Acetylcholine infusion with a dose rate of 11 mg/min was given during 16 min At the isoprenalin administration about 0 20 mg substance was inhaled equivalent to 3—4 inhalations with Isoprenalin N spray (Medihaler Riker) All inhalations were given in the course of three min Eight min after the last inhalation the measurement of cardiac output by means of the direct Fick method was started Expired gas was collected between the 6th to the 16th minute of infusion and between the 3rd to 6th minute of exercise Intravascular pressures were measured and blood samples taken from the 10th to the 14th minute of infusion and the 4th to the 6th minute of exercise

Statistical adviser Gunnar Eklund Ph D For statistics see reference 8 (p 158—159)

FEV 1 0 (% of pred)		FEV % (% of pred)		MVV_{40} (% of pred)		MVV_F (% of pred)		FRC/ TLC (% of pred)	RV/ TLC (% of pred)	Time for He equilibration (min)
b i	a i	b i	a i	b i	a i	b i	a i			
32 3	38 2	60 0	62 5	27 6	35 4	31 0	36 1	120 8	148 6	7
73 3	83 3	95 8	94 4	60 6	70 5	53 6	71 0	118 5	131 7	4
121 0	113 1	104 1	102 7	106 2	114 0	126 9	127 5	80 8	96 6	5
47 4	60 5	86 7	89 3	51 9	56 3	44 9	55 8	96 0	146 2	6
54 5	63 6	65 8	74 7	41 6	48 2	41 8	47 0	125 0	175 0	7
53 3	66 7	54 3	64 2	53 2	72 3	52 9	71 2	133 3	127 6	6
64 0	76 0	73 0	70 5	62 9	78 7	67 0	85 6	93 8	120 0	7
58 2	71 4	80 2	84 0	60 2	72 4	64 0	73 5	115 2	136 8	7
80 4	90 2	86 0	88 6	72 6	85.8	70 4	86 0	118 4	139 6	7
70 2	79 4	69 8	79 1	71 3	86 4	69 6	85 4	129 6	166 7	6
65 5	74 2	77 6	81 0	60 8	72 0	62 2	73 9	113 1	138 9	6 2
121 0	113 1	104 1	102 7	106 2	114 0	126 9	127 5	133 3	175 0	7
32 3	38 2	60 0	62 5	27 6	35 4	31 0	36 1	80 8	96 6	4

Results

Table I gives individual data for lung volumes, relations between certain lung volumes and ventilation capacity before and after bronchodilatation with isoprenalin all as percentages of predicted normal values It appears from the table that all patients except one had signs of hyperinflation measured as the quotient of residual volume and total lung capacity The hyperinflation was generally of a moderate degree

The ventilation capacity measured as $FEV_{1\,0}$ MVV_{40} and MVV_F, was on average reduced to between a half and two thirds of predicted normal values One patient however presented an ordinary ventilation capacity thus differing from the rest of the patients who also had more severe asthma The ventilation capacity of the other nine patients increased after isoprenalin inhalation from a mean value of 59 3 % to 69 9 of predicted normal value, measured as $FEV_{1\,0}$, and from an average of 55 8 % to 67 3 measured as MVV_{40} and from an average of 55 0 % to 68 0 measured as MVV_F

The arterial oxygen saturation at rest was normal in all the patients on an average of 95 1 % During infusion of the pulmonary artery with 11 mg acethyl choline/min the oxygen saturation was significantly reduced to an average of

89 6 % All patients showed reduced oxygen saturation, but the reduction seemed to be slightly more accentuated in the six patients whose asthma was very severe (group C) since its onset (95 4 % to 88 4 %) than in the remaining four (94 8 % to 91 0 %) The difference however did reach a significant level

When the acetylcholine infusion was preceded by inhalation of isoprenalin, practically the same average (89 9 %) of the arterial oxygen saturation was found as at acetylcholine infusion without previous isoprenalin inhalation (89 6 %) The saturation was higher in six patients and lower in four, if infusion was preceded by isoprenalin inhalation

The effect of inhaled isoprenalin on the arterial oxygen saturation after acetylcholine infusion was not related to the assessed degree of severity of the asthma to the ventilation capacity or to the degree of hyperinflation Nor was the increase in ventilation capacity by inhaled isoprenalin related to the decrease in oxygen saturation during acetylcholine

The arterio venous oxygen difference decreased to an average of 40 2 ml/l during infusion of acetylcholine from a mean value of 44 1 ml/l at ordinary resting conditions When the acetylcholine infusion was preceded by isoprenalin inhalation the difference decreased still more to a mean of 35 5 ml/l which was significantly lower than the control value Oxygen uptake cardiac output and stroke volume all increased significantly during acetylcholine infusion from an average of 213 to 242 ml/min 4 9 to 6 5 l/min and 67 to 79 ml respectively, compared with control conditions For the latter parameters there were, generally, no noteworthy differences between the values during acetylcholine infusion and acetylcholine infusion preceded by isoprenalin inhalation

The heart rate increased significantly in the patient series from an average of 74 8 beats/min to 82 6/min during acetylcholine infusion When the infusion was preceded by isoprenalin inhalation the heart rate increased to 84 8/min

All patients had normal pressures of the pulmonary circulation at rest The systolic pressure in the pulmonary artery was on an average 22 4 mm Hg (15—28), and the mean pressure was 14 5 mm Hg (10—20) During acetylcholine infusion, with or without preceding isoprenalin inhalation, there was no significant change of the pressure in the pulmonary artery

During physical work, 200 and 400 kpm/min for women and 300 and 600 kpm/min for men respectively, the systolic pressure in the pulmonary artery increased during the first load to 34 5 mm Hg (22—53), and the mean pressure to 23 4 mm Hg (15—34)

During the first load a systolic pressure exceeding 32 mm Hg was registered in six out of ten patients, and, also in six out of ten patients, a mean pressure of more than 22 mm Hg

The higher load was performed by eight patients Their systolic pressure in the pulmonary artery was on average 35 9 mm Hg (26—45), and the mean pressure 23 8 mm Hg (15—28) Five out of these eight patients had a systolic pressure exceeding 32 mm Hg, and six a mean pressure above 22 mm Hg

The mean pressure of the pulmonary capillaries (PCV) was within normal limits in all patients at rest (mean 7 9 range 4—11 mm Hg) as well at at work (mean 10 1 range 7—12 mm Hg) at the highest fulfilled load

The pulmonary vascular resistance (R) expressed in mm Hg/l/min $\frac{(P_{PA}-P_{PCV})}{Q}$ was determined at rest as well as during the highest performed work load The resistance at rest was on average 1 48 (0 50—3 05) and at work on an average 1 44 (0 23—4 26) The pulmonary vascular resistance index (R) expressed as mm Hg/l/min m BSA $\frac{(P_{PA}-P_{PCV})\ BSA}{Q}$ was also calculated for the same persons This index was at rest mean 2 52 (0 85—4 76) and at work mean 2 43 (0 35—6 22)

The pressure in the systemic artery does not seem to have been influenced neither by acetylcholine infusion nor by isoprenalin inhalation to any considerable extent The mean arterial oxygen saturation was at the lowest load 94 8 % (89 8—99 5 %) and at the highest load 95 7 % (90 9—97 6) values which should be compared with a mean value at rest of 95 1 % (92 3—98 9 %) The results are assembled in table II

Discussion

In an earlier investigation on the effect of acetylcholine infusion on the pulmonary circulation in asthma patients (10) we found a significant reduction of SaO Such an effect would support the hypothesis that the SaO reduction caused by acetylcholine could be related to a changed ventilation/perfusion ratio In the present investigation however we found no influence of isoprenalin inhalation on the reduction of SaO during acetylcholine infusion The infusion caused a similar reaction with or without preceding isoprenalin inhalation namely a higher cardiac output and a higher oxygen uptake reduced AVD and essentially unchanged mean pressures in the systemic and the pulmonary circulations There may be several reasons for the increase in cardiac output and oxygen uptake during the pharmacological tests and uneasiness of the patient may have been a contributory factor

The essential finding for the problem of the present study that the reduction of arterial oxygen saturation following acetylcholine infusion is not significantly influenced by isoprenalin inhalation even if isoprenalin is administered in considerable quantity can be interpreted in different ways It is not definitely proved that the patients have absorbed the intended quantity of isoprenalin As we had trained the patients with the inhalation method in advance and at the test carefully supervised the inhalations we consider these to have been performed in an optimal way

At the spirometric tests the patient series showed an increase of the maximal voluntary ventilation (MVV) after isoprenalin inhalation This increase is of the same order of magnitude as that obtained earlier (9) in another comparable study using the same methods The increase in MVV after isoprenalin inhalation can be explained if the inhalation only caused already ventilated pul

TABLE II Individual data and mean values from pressure and flow determinations during right

Pat	Sex	Age	Oxygen saturation (per cent)						
			Br A					PA	
			R	A	A+I	W_1	W_2	R	A
A S	♂	56	93 6	84 5	84 9	89 8		69 2	66 6
R O	♂	54	92 3	89 7	91 2	96 1	97 6	71 0	57 8
H H	♂	49	97 3	95 4	93 9	95 9	96 7	73 5	75 4
K A	♂	45	93 9	90 3	89 1	95 3	96 1	70 6	66 7
B E	♀	54	93 2	84 5	84 3	93 9		67 8	62 9
I N	♀	44	94 7	87 4	89 6	90 5	90 9	69 7	67 2
M S	♀	43	94 4	91 0	89 1	94 6	96 1	62 7	65 3
B F	♀	34	97 0	86 0	86 3	96 3	97 3	76 3	70 1
U R	♀	33	98 9	92 9	96 0	96 0	94 1	78 2	75 5
B F	♀	24	95 8	94 6	94 7	99 5	96 5	71 1	70 3
Mean		43 6	95 1	89 6	89 9	94 8	95 7	71 0	67 8
Highest		56	98 9	95 4	96 0	99 5	97 6	78 2	75 5
Lowest		24	92 3	84 5	84 3	89 8	90 9	62 7	62 9

Pat	Sex	Age	Oxygen uptake (ml/min) STPD					Cardiac output	
			R	A	A+I	W_1	W_2	R	A
A S	♂	56	236	266	252	965		6 2	9 6
R O	♂	54	107	157	174	849	1,913	2 5	2 5
H H	♂	49	241	293	260	896	1 420	5 3	7 7
K A	♂	45	311	323	304	1 051	1 530	6 1	6 3
B E	♀	54	181	204	187	602		3 6	4 7
I N	♀	44	190	236	210	749	1 089	4 2	6 5
M S		43	227	238	244	791	1 168	3 9	5 0
B F		*34*	*212*	*238*	*234*	*721*	*1 050*	*6 1*	*8 9*
U R	♀	33	192	232	217	659	977	5 3	7 8
B F	♀	24	234	234	205	701	1 058	6 0	6 1
Mean		43 6	213 1	242 1	228 7	798 4	1 275 6	4 9	6 5
Highest		56	311	323	304	1 051	1 913	6 2	9 6
Lowest		24	107	157	174	602	977	2 5	2 5

Br A = brachial artery

PA = pulmonary artery

AVD = arterio venous oxygen difference

R = rest A = during acetylcholine infusion A+I = during acetylcholine infusion after isoprenalin inhalation. W_1 = first work load W_2 = second work load

heart catheterization before and during acetylcholine infusion and isoprenalin inhalation

			AVD (ml/l)				
A+I	W_1	W_2	R	A	A+I	W_1	W_2
64 7	43 7		38 3	27 6	24 0	74 6	
71 2	50 0	44 1	43 3	63 9	40 2	96 6	117 7
76 2	52 2	51 0	45 2	38 3	32 4	81 9	89 2
68 3	51 5	46 8	50 7	51 3	44 5	96 9	111 7
63 6	38 6		49 7	43 3	41 2	112 4	
70 6	45 7	39 2	44 8	36 1	36 0	81 4	96 3
67 9	44 1	37 7	58 9	47 4	40 2	97 4	116 6
69 9	55 5	51 9	34 9	26 7	27 3	70 3	78 5
76 5	55 5	51 3	36 0	29 6	34 8	71 2	75 9
73 1	52 4	41 6	38 9	38 2	34 8	76 9	92 5
70 2	48 9	45 5	44 1	40 2	35 5	86 0	97 3
76 5	*55 5*	*51 9*	*58 9*	*63 9*	*44 5*	*112 4*	*117 7*
63 6	38 6	37 7	34 9	26 7	24 0	70 3	75 9

(l/min)			Stroke volume (ml)				
A+I	W_1	W_2	R	A	A+I	W_1	W_2
10 5	12 9		65	98	102	105	
4 3	8 8	16 3	31	31	50	71	103
8 0	10 9	15 9	80	102	96	111	96
6 8	10 8	13 7	77	85	81	93	91
4 5	5 4		40	48	56	48	
5 8	9 2	11 3	66	76	80	79	77
6 1	8 1	10 0	76	79	87	87	86
8 6	10 3	13 4	68	83	57	79	80
6 2	9 3	12 9	84	108	79	96	99
5 9	9 1	11 4	83	80	76	118	85
6 7	9 5	13 1	67 0	79 0	76 4	88 7	89 6
10 5	12 9	16 3	84	108	102	111	103
4 3	5 4	10 0	31	31	50	48	77

Table II (cont)

Pat	Sex	Age	Heart rate (beats/min)					Pressures (mm Hg)	
								PA	
								Systolic	
			R	A	A+I	W_1	W_2	R	A
A S	♂	56	95	97	102	122		24	25
R O	♂	54	79	80	85	123	158	18	16
H H	♂	49	66	75	83	98	142	21	23
K A	♂	45	79	74	83	116	150	26	22
B E	♀	54	89	97	95	113		28	22
I N	♀	44	63	85	72	116	145	24	32
M S	♀	43	53	63	70	93	115	28	31
B F	♀	34	89	107	103	130	166	20	25
U R	♀	33	63	72	78	96	130	20	20
B F	♀	24	72	76	77	113	134	15	15
Mean		43.6	74.8	82.6	84.8	112.0	142.5	22.4	23.1
Highest		56	95	107	103	130	166	28	32
Lowest		24	53	63	70	93	115	15	15

Pat	Sex	Age	Pressures (mm Hg)						
			PA					Br A	
			Mean					Systolic	
			R	A	A+I	W_1	W_2	R	A
A S	♂	56	15	17	13	34		134	140
R O	♂	54	11	11	10	22	27	130	145
H H	♂	49	14	16	19	22	27	155	120
K A	♂	45	16	17	14	23	27	158	145
B E	♀	54	20	18	15	31		185	185
I N	♀	44	15	20	14	25	28	140	155
M S	♀	43	16	18	14	23	26	200	195
B F	♀	34	15	17	17	24	23	140	145
U R	♀	33	13	13	13	15	15	125	125
B F	♀	24	10	9	11	15	17	118	118
Mean		43.6	14.5	15.4	14.0	23.4	23.8	148.5	147.3
Highest		56	20	20	19	34	28	200	195
Lowest		24	10	9	10	15	15	125	118

			Diastolic				
A+I	W_1	W_2	R	A	A+I	W_1	W_2
20	53		11	13	9	25	
17	33	40	8	9	7	17	17
25	31	42	9	10	13	14	18
22	34	45	10	10	9	13	17
21	39		14	12	12	22	
22	35	35	10	12	8	18	20
25	44	40	9	9	7	5	6
25	32	32	10	11	11	15	15
23	22	26	9	9	9	10	10
18	22	27	7	7	8	10	12
21 8	34 5	35 9	9 7	10 2	9 2	14 9	14 4
25	53	45	14	13	13	25	20
17	22	26	7	7	7	5	6

			Diastolic				
A+I	W_1	W_2	R	A	A+I	W_1	W_2
130	195		78	80	72	95	
152	180	195	80	95	88	98	110
125	180	215	95	70	75	100	105
140	180	*190*	*105*	*90*	*94*	*110*	*115*
180	200		112	108	108	105	
130	170	180	90	95	75	90	95
190	225	230	90	85	85	95	90
145	170	175	93	90	90	105	105
135	140	160	70	65	70	70	80
120	140	150	75	65	68	80	75
144 7	178	186 9	88 8	84 3	82 5	94 8	96 9
190	225	230	112	108	108	110	115
120	140	150	70	65	68	70	75

Table II (cont.)

Pat.	Sex	Age	Br. A.				
			Mean				
			R	A	A+I	W_t	W_a
A S	♂	56	100	102	95	132	
R O	♀	54	104	115	112	132	145
H H	♂	49	120	80	95	120	145
K A	♀	45	124	110	116	135	145
B E	♀	54	144	138	140	150	
I N	♀	44	110	125	100	120	125
M S		43	125	130	130	145	135
B F	♀	34	115	110	110	125	130
U R	♀	33	90	80	90	95	110
B F	♀	24	96	88	85	100	106
Mean		43.6	112.8	107.8	107.3	125.4	130.1
Highest		56	144	138	140	150	145
Lowest		24	90	80	85	95	106

monary areas to become even better ventilated and if zones excluded from ventilation were not affected to any noticeable extent, for instance zones shut off by mucous obstruction of the bronchioli. Anyway, the isoprenalin apparently did not significantly influence the ratio between ventilation and perfusion in earlier underventilated areas.

It is also possible that the acetylcholine infusion in asthma patients leads mainly to a *shunting* of the pulmonary blood flow through vessels not passing alveoles (anatomic shunting). Yet earlier investigations do not seem to support the hypothesis that the whole reduction of SaO_2 at acetylcholine infusion could be explained by anatomic shunting. Fritts et al. (5) were not able to show any reduction of the arterial oxygen saturation in normal persons after acetylcholine infusion (0.5 mg/min.) into the pulmonary artery. Schlant et al. (13), however, found that in patients with diseases other than obstructive pulmonary disease acetylcholine infusion (mean value 2.47 mg/min.) gave a reduction of SaO_2 from an average of 94.9 per cent at rest to 88.7 per cent at infusion. Chidsey et al. (4) in patients with pulmonary emphysema found that acetylcholine infusion in the right atrium lowered the arterial oxygen saturation in nine out of 13 cases. The authors considered the reduction to be best explained by the fact that acetylcholine infusion dilated vessels which were contracted by hypoxia and consequently increased the perfusion to badly ventilated pulmonary areas.

Niden et al. (12) found in dogs the arterial oxygen saturation to decrease during acetylcholine infusion. This effect

PCV		Pulmonary vascular resistance		Pulmonary vasc. resist index	
Mean		$\frac{P_{PA}-P_{PCV}}{Q}$		$\frac{(P_{PA}-P_{PCV})}{Q}$ BSA	
R	W	R	W	R	W
5	12	1 61	1 71	3 12	3 32
4	11	2 8	0 98	4 76	1 67
11	12	0 57	0 94	1 14	1 88
7	11	1 48	1 17	2 99	2 36
9	8	3 05	4 26	4 45	6 22
8	10	1 67	1 59	2 61	2 48
11	11	1 28	1 50	2 32	2 72
7	7	1 31	1 12	2 10	1 79
10	12	0 57	0 23	0 87	0 35
7	7	0 5	0 88	0 85	1 50
7 9	10 1	1 48	1 44	2 52	2 43
11	12	3 05	4 26	4 76	6 22
4	7	0 50	0 23	0 85	0 35

was reduced but not eliminated if the animal inhaled a gas mixture containing 25—30 % oxygen The authors inferred that the acetylcholine principally influenced the ventilation/perfusion ratio but, in addition, gave rise to a local physiologic or anatomic blood shunting

A problem arises as to whether acetylcholine, administered as in our investigation, has any essential bronchoconstrictive effect Some of our patients experienced a feeling of heavier breathing and a slight cough during the first minutes of the acetylcholine infusion These symptoms were temporary, and when the measurements began, all patients were symptom free With comparable patient material the maximal expiratory flow rate (MEF) in l/min was determined partly before, and partly after infusion of acetylcholine The material consisted of 25 patients on the one hand those accounted for in an earlier investigation (8 10) in addition to six from the present patient series The MEF value before infusion was measured with the patient in sitting position, after infusion with the patient supine A series of three measurements were done before as well as after the infusion the highest value

TABLE III Mean value standard deviation and standard error of mean of maximal expiratory flow rate in asthma patients before and after acetylcholine infusion

	Mean	S D	S E M	Range
Sitting before	240 0	115 8	23 6	120—560
Recumbent after	215 0	109 0	22 3	100—530

TABLE IV Mean value standard deviation and standard error of mean of maximal expiratory flow rate in 19 normal individuals in sitting and recumbent position

	Mean	S D	S E M	Range
Sitting	*531*	*95 0*	*21 8*	*440—750*
Recumbent	511	91 6	21 0	390—750

from each series forms the basis of table *III*

The reduction of the MEF value, which on average, was obtained with the patient recumbent after the infusion compares well with the one found in controls consisting of 19 normal individuals without acetylcholine infusion (table IV)

If the maximum expiratory flow is used as an index for the degree of bronchoconstriction it is reasonable to conclude that acetylcholine did not cause significant bronchoconstriction

It is interesting that in our patient series there was no significant change in arterial oxygen saturation during physical exercise compared with conditions at rest In our present series the exercise test was preceded by the pharmacological test with acetylcholine and isoprenalin This probably was not a cause of error since in an earlier investigation on patients with asthma bronchiale (Irnell) in which a pharmacological test was not performed, the arterial oxygen saturation was essentially the same at rest as at work Irnell and Swartling (11) in an earlier investigation showed that the MEF in asthma patients increases during physical exercise in the sitting position The pulmonary perfusion increase during exercise thus seemed to occur essentially in pulmonary areas where the ventilation was also increased

The acetylcholine infusion seems to have given a perfusion increase in pulmonary areas where an increased ventilation was not possible to achieve in spite of isoprenalin supply Thus, different areas or sections of pulmonary vasculature may be influenced by acetylcholine infusion and by the increased cardiac output in physical exercise

Isoprenalin inhalation in our series of patients did not prevent the decrease of arterial oxygen saturation during acetylcholine infusion However, this fact does not prove that the acetylcholine effect is caused by a perfusion increase in non alveolar lung vessel sections (anatomic shunting) An additional or alternative explanation is that isoprenalin inhalation does not give increased ventilation to those hypoventilated alveolar areas which vasodilate during acetylcholine infusion

Summary

In a series of patients with asthma bronchiale in free interval, inhalation of isoprenalin did not prevent decrease of the arterial oxygen saturation caused by infusion of acetylcholine in the pulmonary artery During acetylcholine infusion a decrease of arterial oxygen saturation from 95 1 % to 89 6 % was obtained When the infusion was preceded by isoprenalin inhalation, the corresponding decrease was 89 9 % During muscular exercise there was no decrease

in arterial saturation The result neither corroborates nor disproves the idea that the acetylcholine effect is a vasodilatation of pulmonary vessels originally constricted because of proximity to hypoventilated alveoli

Acknowledgement

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Ergotism, Arteriospastic Disease and Recovery, Studied Angiographically

By

S Fagerberg, H Jorulf and C G Sandberg

Ergotamine is considered a specific remedy for attacks of migraine If properly administered it can relieve most attacks of migraine, as first demonstrated in Sweden in the early 30 s by Ask Upmark (2) This action seems to be due to a specific constrictor effect upon the extracranial blood vessels which are dilated and pulsate during the attacks whereas the main toxic side effect is due to adrenergic blocking with ensuing peripheral vasoconstriction leading to gangrene in the extremities In our medical department we have repeatedly seen the vasoconstrictor action not only on the arteries of the lower extremities but also on the coronary arteries according to Ask Upmark (3) these preparations should never be used in patients ≧ 50 years of age

In the present case we had the opportunity to demonstrate this reversible vasconstrictor effect radiologically by angiography

Case report

A V a married woman, aged 42 was admitted to our clinic on the 13th of March 1967 because of migraine Mother died when 48 from stroke she was said to have had headaches for several years, as had some of her sisters One maternal cousin had headaches of migraine type as well and died from renal insufficiency (phenacetin-kidney?) Father alive aged 75 Family history otherwise of no interest Normal deliveries in 1947, 1949 and 1954 Earlier history she is said to have had occasional attacks of Raynauld type as a child, and also rare attacks as an adult When 12 erythema nodosum (tuberculous etiology)

Since 1957 migraine with headaches nausea vomiting hypersensitivity to light and to noise cannot use TV and cannot stand noise The headaches occur throughout the head except for the week preceding the menstruation when they are on one side Micropsy has occasionally been noticed When the pains are very severe a compression around the head may help Since 1959 the patient has used Syncapton® suppositories daily (ergotamine tartrate 2 mg diphenhydraminteoklat 50 mg and caffeine 0 2 g) During the two last years she has experienced peripheral coldness and suffered from intermittent claudicatio

Routine examination on admission General condition fair Pulse rate 72 B P 140/85 There were no inverted nipples The forehead was warm whereas hands and feet were cold Ophthalmologic examination revealed a bilateral central cataract This may be a

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Fig 1 Angiography at admission considerable narrowing of distal parts of both superficial femoral arteries with collateral circulation

congenital defect but might have been induced by ergotamine Heart lungs, abdomen and general neurology normal Laboratory tests of blood and urine were normal

EEG showed unspecific episodes of the ... No paroxysmal or focal activity was registered Left sided carotid angiography showed normal conditions

After admission angiography of the femoral arteries was performed after insertion of a catheter into the lower part of the abdominal aorta via the femoral artery A 7—8 cm length of the distal parts of both superficial femoral arteries was very narrow with circulation through dilated collateral vessels fig 1 There were no changes in the lower part of the abdominal aorta and the iliac arteries The angiography was repeated five and nine weeks later These examinations both showed a marked regression with only 2 cm of each superficial femoral artery slightly narrowed The collateral circulation had almost subsided (fig 2

After admission to the hospital no ergotamine preparations were given and thereafter there were no clinical symptoms of arterial insufficiency in the legs

Discussion

In 1936 Yater and Cahill (14) recorded a case of bilateral gangrene of the feet due to administration of ergotamine tartrate In one leg they performed a thorium dioxide arteriogram, the injection being into the popliteal artery They found narrowed arteries, peripherally occluded, with collaterals This was the first case to be studied angiographically In two cases of ergotism Johnsson in 1962 (10) described contracted arteries as verified by angiography in one case the external iliac



Fig 2 Angiography five weeks after admission only slight narrowing of both sup fem. arteries. Collateral circulation almost subsided.

and femoral arteries were symmetrically involved and in one case the femoral vessels slightly narrowed in their upper and middle parts but tightly contracted in their lower parts with evidence of collateral circulation and dilated vessels He showed angiographically that contractions disappeared after withdrawal of the ergotamine preparations Allen et al in 1962 (1) showed by aortography in one patient with ergotism a spasm of the external iliac artery with almost complete disappearance of the spasm after withdrawal of the ergot preparation Kramer et al in 1965 (11) found complete obstruction with collateral circulation in both axillary arteries after selective injection into the innominate and left subclavian arteries in a patient given an ergot preparation

The present case represents chronic ergotamine poisoning with fairly rapid recovery from arterial insufficiency in the legs after withdrawal of the drug A striking feature is the symmetrical distribution of the vasoconstriction of peripheral arteries

Ask Upmark (5) has called attention to the frequent occurrence of cold hands and feet in patients with migraine in vivid contrast to their warm forehead This reduced temperature of the skin of hands and feet may call for a warning against the uncritical use of ergotamine preparations in migraine

Ergotamine should be prescribed with

great caution and only for occasional, very severe attacks of migraine in people 50 years or older We had a few years ago in our clinic a woman in her 50 s who died from uremia because of damage caused to her renal vessels by abundant use of ergotamine in migraine

Occasionally, ergotamine has been used for other purposes as well Thus, last year we had in our clinic a young girl student (aged 23) with hemophilia who for several years had been taking ergotamine, of late in daily injections, obviously in order to reduce the hemorrhage of her menstruations This girl had very cold hands and feet and the peripheral pulses were not palpable Because of her hemophilia we did not want to make arteriographies in her case she improved considerably whilst in the hospital but there could be no doubt about her ergotism

In a recent paper Brohult et al (6) have described a case of multiple arterial thrombosis in a woman aged 35, who was prescribed ergotamine because of headaches She died from thrombosis of her carotid artery However oral contraceptives in this case also had been used so we would rather consider the ergotamine innocent in this case and the contraceptives the culprit (cf 4)

Summary

One case of chronic ergotamine poisoning with recovery has been studied by angiography In such a case it would seem wise to avoid all ergotamine preparations and also oral contraceptives and smoking

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Complications Following Resuscitation

By

NARVE MOE

Since external cardiac massage was introduced in 1960 (16), this method combined with artificial ventilation has become widely used The procedures are occasionally accompanied by complications

Related to the chest massage, the most common complications are fractures of the ribs (3, 4, 5, 7, 10, 11, 12, 13, 14, 18, 21, 22, 24), fracture of the sternum (7, 11, 13, 21, 24), rupture of the liver (4, 7, 11, 13, 14, 20), rupture of the spleen (7), bone marrow emboli (4, 12, 13, 14), hemothorax (3, 4 21, 24), pulmonary hemorrhage (7, 8), hematoma (18) or rupture (21, 25) of the heart, hemorrhage of the pancreas (7, 8), and retroperitoneal hemorrhage (7)

Intracardial injection of pharmacological agents may cause pneumothorax (14) or hemopericardium (3, 4)

Improper artificial ventilation in the newborn infant may result in pulmonary interstitial emphysema, pneumomediastinum pneumothorax, and pneumoperitoneum (6, 9, 15, 19, 26) Pneumothorax in an adult with lung emphysema is also described (7)

Between 1st of January 1964 and 1st of April 1967 16 cases with complications due to resuscitation efforts have been autopsied in the Department of Pathology, Ulleval Hospital During the same period the total number of autopsies was 5 679

Rib fractures and fracture of the sternum are common lesions following external cardiac massage These complications are reported only when combined with other injuries

Case reports

Case 1

An 8 hour old male infant was born asphyctic, and terminally external cardiac massage and artificial respiration with the mouth-to-mouth/nose method were performed by a doctor for an unknown length of time Autopsy findings showed prematurity atelectatic lungs and hyaline membrane disease There was a tear in the liver of 1 cm length and approximately 15 ml of blood in the peritoneal cavity

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Case 2

A 17 hour old male infant was born ten weeks before term The child seemed well immediately after birth Seventeen hours later he developed severe cyanosis External cardiac massage and artificial respiration by the mouth to-mouth/nose method were performed by a doctor for ten minutes Autopsy findings showed prematurity, mitral stenosis and atresia of the aorta a tear in the liver of 1 cm length and approximately 20 ml of blood in the peritoneal cavity

Case 3

A 31 hour old male infant was born asphyctic and terminally external cardiac massage and artificial respiration with the mouth to-mouth/nose method were performed by a doctor for an unknown length of time Autopsy findings showed prematurity, atelectatic lungs and hyaline membrane disease There was a rupture in the liver of 6 cm length and small amounts of blood in the peritoneal cavity

Case 4

An 18 month old male infant developed a gastro-enteritis, and two days after the onset the infant was found unresponsive in his bed at home External cardiac massage and artificial respiration by the mouth to-mouth nose method were performed during the transport by an ambulance man for approximately 15 minutes Autopsy findings showed acute enteritis cerebral edema and fatty infiltration of the liver The stomach showed a rupture of 2 cm diameter located in the lesser curvature right distal to the cardia There was no tissue reaction around the lesion There was pneumoperitoneum and complete collapse of the lungs due to a tension pneumothorax

Case 5

A 43 year old man was taken ill at home with dizziness nausea and vomiting He collapsed and was brought to the hospital emergency room where a doctor performed external cardiac massage and artificial respiration for an unknown length of time Autopsy findings showed multiple pulmonary emboli, thrombi in the periprostatic vein plexus and a tear in the spleen of 4 cm length There was no hemorrhage

Case 6

A 46-year old man with a previous history of coronary disease collapsed in the street just outside the hospital He was brought to the emergency room where external cardiac massage and intratracheal intubation and positive pressure ventilation were performed by the doctor for about 20 minutes. Autopsy findings showed severe coronary atherosclerosis, cardiac hypertrophy, and old myocardial infarction, fracture of the third through sixth ribs on the left side, and a fracture of the sternum There was fatty infiltration of the liver with a tear of 7 cm length and about 100 ml of blood in the peritoneal cavity

Case 7

A 57 year old man developed chest pain and collapsed at home During the transport to the hospital external cardiac massage and artificial respiration by the mouth to-mouth method were performed by the ambulance man In the hospital emergency room cardiac massage intratracheal intubation and positive pressure ventilation were given by a doctor for an unknown length of time Autopsy findings showed a coronary occlusion a rupture in the liver of 2 cm length, and approximately 400 ml of blood in the peritoneal cavity

Case 8

A 62 year old man developed abdominal pains and shock at his office He was brought to the hospital where aortography showed an abdominal aneurysm An operation with dacron transplantation was performed After the operation he was unconscious and anuric The first post-operative day he developed cardiac arrest External cardiac massage was performed by a doctor for a few minutes, and the heart started to beat again However he got gradually worse and died four hours later Autopsy findings showed an intact

tran.plantate in the aorta fracture of the fourth and fifth ribs at the r ght s de ap prox mately 00—800 ml of blood in the per oneal cav ty and a rupture in the l ver of 7 cm length The rupture was filled w th coagulated blood

Case 9

A 64 year old man de eloped chest pain and collapsed at home During the transport to he hosp tal external card ac massage as performed by an ambulance man for ap p oximately 15 minutes Autopsy f nd ngs howed coronary atherosclerosis card ac hy er rophy old myocard al infarct on mult p le fractures of the ribs and a tear n the l er of 4 cm length There as no hemor hage from the l ver

Ca e 10

A 65 year old man de eloped chest pa n at home He was brought to the hosp tal where ECG sho ed an anterior myocard al infarc t on A fe v hours after the adm ss on he collapsed and external card ac massage was performed by a doctor for about 15 minutes Adrenal n as njected intracard ally Au topsy find ngs sho ed coronary occlus on myocard al infarction and rupture of the heart There was approximately 20 ml of blood outs de the per card um n relat on to two njec on openings

Case 11

A 68-year old man developed chest pain and ollapsed at home During the transport to the ho p tal external card ac massage was performed by an ambulance man for approxima ely ten m nutes Autopsy findings sho ed a myocardial infarct on mult ple f actures of the ribs patches of hemorrhage in the parenchyma of the lungs and a hem orrhage of 50 to 70 ml of blood around he portal ve n

Ca e 12

A 63 year old woman as n the ho p tal be ng trea ed w th osteosynthesis for fractures n the left ankle T o weeks after the opera t on she suddenly collapsed House doctors



F g 1 Case 13 Rupture in the l er

ere called and performed external card ac massage ntratracheal ntubat on and pos t e pressure vent lation for three hours Autopsy f ndings showed a large pulmonary embolus a thrombus in the femoral ve n fracture of the sternum fracture of the sec ond and th rd r bs on the r ght s de and of the second through s xth r bs on the left s de The spleen sho ed a rupture of 4 cm length and there was approximately 3 000 ml of blood n the per toneal cav ty

Case 13

A 0 year old oman de eloped dyspnea and chest pain at home ECG in the ho p tal ho ed a posterior myocard al infarct on The day after the adm ss on she suddenly collapsed External card ac massage and artific al re p ration by the mouth to-mouth me hod ere performed by a doctor for 20 n nutes Autopsy findings showed a coronary occlus on a myocard al nfarct on w th rup ture of the heart a rup ure n the l er of



Fig 2 Case 13 Mucosal tears at the esophagogastric junction (Mallory Weiss syndrome)

6 cm length (fig 1) and about 50 ml of blood in the peritoneal cavity In the stomach some tears of the mucosa were found The tears were up to 4 cm in length longitudinal in position and confined to the lesser curvature close distally to the esophagogastric junction (fig 2)

Case 14

A 71 year old woman developed shortness of breath and chest pain at home She collapsed immediately after the admission to the hospital External cardiac massage was performed by a doctor for approximately ten minutes Autopsy findings showed a myocardial infarction There was fatty infiltration of the liver which showed a tear of 2 cm length and multiple deep ruptures In the peritoneal cavity about 400 ml of blood was found

Case 15

A 78 year old woman developed chest pain at home She was brought to the hospital emergency room where she collapsed External cardiac massage and artificial respiration by the mouth-to-mouth method were performed by two nurses for 15 minutes Autopsy findings showed a coronary occlusion myocardial infarction, multiple fractures of the ribs, and about 300 ml of blood retroperitoneally

Case 16

An 82 year old woman developed shortness of breath with chest pain at home Shortly after the admission to the hospital she collapsed. External cardiac massage, intratracheal intubation and positive pressure ventilation were performed by a doctor for about 20 minutes During the artificial ventilation a cervical, subcutaneous emphysema was observed Autopsy findings showed severe coronary atherosclerosis, old myocardial infarctions multiple fractures of the ribs, a rupture in the spleen of 5 cm length, and 300 ml of blood in the peritoneum In the trachea 4 cm distal to the vocal cords a tear in the mucosa of 1 cm length was found (fig 3) There was a severe pneumomediastinum (fig 4)

Comment

The combination of external cardiac massage and artificial respiration can keep alive a victim of cardiac and respiratory arrest for a reasonable period The experiences reveal both the benefits and the hazards of resuscitation and the need for training of medical and non medical personnel In this report of 16 cases with resuscitative complications, the procedure was performed by a doctor in 11 cases by the nurses in one and by an ambulance man in three cases In one case an ambulance man



Fig 3 Case 16 Tear in the tracheal mucosa



Fig 4 Case 16 Pneumomediastinum

started the resuscitation which was continued by a doctor

The present study comprises complications of external cardiac massage (table I) and of artificial respiration (table II) Most of the complications are probably due to unsatisfactory technique The most common complication is rib fractures which may occur when pressure is applied to the side of the midline (1 4 14) Lacerations of the liver are often due to compression over the xiphoid process at the tip of the sternum (1) Noticeable in this report are the two cases with rupture in a fatty infiltrated liver The danger of lacerating the liver is greater in children because of the higher position of the liver under the lower sternum (1) Three of the nine cases with rupture of the liver in this report were children

Exhaled air ventilation (mouth to

TABLE I Complications of external cardiac massage

Complication	No of cases
Fractured ribs combined with other lesions	7
Fractured sternum combined with other lesions	2
Ruptured liver	9
Ruptured spleen	3
Retroperitoneal hemorrhage	2
Pulmonary hemorrhage	1

TABLE II Complications of artificial respiration

Complication	No of cases
Mucosal tears in the stomach	1
Ruptured stomach	1
Pneumothorax	1
Tracheal lesion with pneumomediastinum	1

mouth, mouth to-nose ventilation) frequently causes distension of the stomach This occurs most often in children (1) and specially in newborn infants who have not been breathing previously (26) In case 4 the rupture of the stomach most probably was due to exhaled air ventilation and not a result of gastroenteritis because of the absence of tissue reaction around the lesion Mucosal tears at the esophagogastric junction the Mallory Weiss syndrome (17) can be produced in cadavers by blowing air into the stomach at an intragastric pressure of 150 mm Hg (2)

Damage to the respiratory mucosa may predispose to subcutaneous emphysema pneumomediastinum and pneumothorax Subcutaneous emphysema as a complication of endotracheal intubation has previously been described (23)

Summary

External cardiac massage and artificial respiration have been found to entail some serious complications if improperly applied During the past 3 1/4 years autopsies of 16 cases with complications due to resuscitation efforts have been performed in this laboratory (tables I and II) The material consists of four male infants, seven men and five women Rib fractures and fracture of the sternum are reported only when combined with other injuries

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Coexistent Sarcoidosis and Hyperparathyroidism

By

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Since the first description of hypercalcaemia in sarcoidosis (21), it has been repeatedly shown that this condition and hyperparathyroidism are closely similar biochemically and clinically and may be hard to differentiate (9, 14, 32 35) The simultaneous occurrence of both diseases has been reported several times since 1958 (4 6, 13, 15, 19, 22 34) and it has recently been suggested that this difficult double diagnostic situation occurs too often to be attributable to chance (13)

This paper describes the clinical course and discusses the biochemical findings in a patient with sarcoidosis, in whom hypercalcaemia and marked hypercalcuria disappeared after removal of a parathyroid adenoma

Case report

During a routine X ray examination of the chest bilateral hilar and paratracheal lymph node enlargement was found accidentally in a 17 year old youth who was completely asymptomatic and who apart from tonsillectomy in 1952 had no prior admissions Because of the abnormal X ray he was admitted on October 25 1966 to the Department of Thoracic Medicine of this hospital

Here the presumptive diagnosis of sarcoidosis was confirmed by mediastinal lymph node biopsy There were neither peripheral lymph node enlargement nor signs of pulmonary cutaneous hepatic renal or ocular involvement. Pulmonary function tests bronchoscopy and biopsies from skin muscle liver and bronchial mucosa were normal Routine examination, however revealed hypercalcaemia and hypercalcuria and because of these abnormalities he was transferred to this medical department on November 7 1966 for further study

On admission the patient looked well he had no complaints and questioning elicited no hypercalcaemic symptoms On restricted calcium intake (general ward diet minus milk and cheese) there was persistently raised serum calcium between 12 0 and 14 3 mg/100 ml and renal calcium excretion was constantly high on an average 638 mg/24 hours as shown in fig 1 Serum inorganic phosphate was 2 3—3 2 mg/100 ml most determinations being just below our lower normal value of 2 7 mg/100 ml Despite this the urinary excretion of phosphate was increased to 1 000—1 500 mg/24 hours and the percentage of tubular reabsorption of

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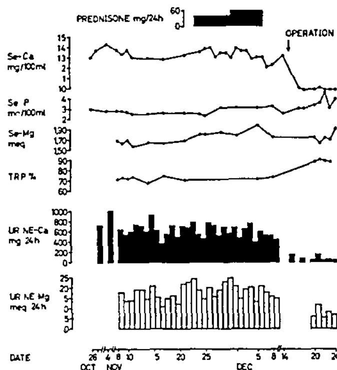


Fig 1 Serial observat o s on the levels of seru n calcium i organ c phosphate magnes um and ur na ry calcium and magnes um excre t o *before and af er operation for* parathyro d adenoma TRP c = percentage of tubular real sorp t on of f l ered phosphate

f l ered p h p l a e as a ord ngly co istantly subnor al b ee i f and 75 % Rad o-graph surve s of the keletal ys en sl o d for sar o d or hyperpara hyrotox c bo e les ons and an p elogran as norr al

The hypercal aem a as unchanged after he ad n ra o of predn sone 10 mg four s la l f r s lays th s is pur od f a n f l ort sone d fferen ial tes h cl p rf m d n ery pat ent h hyperca l cean a In es f he es ablished d agnos s f sar d s s t as ns dered ne e sary to ntinue l pred son for an her s x days v th the da dose ra d o 60 m H per calcaem a and hypercal ur a rema ed un chan ed h he da ly dose rais d to 60 as shown in f l the result a thus s ll unequ ocal and s r ngly sugg s d the s ltaneous pr sen e of hyperparathyro d

On December 15 1966 the neck a explored and a parath yro d adenoma n eas ur ng 18 × 12 × n n and loca ed at the *lo ver pole of the right l be of the thyro d* gland as ren oved The left nferior pa rathyro d gland as dentif ed and app ar d normal M cro cop c epam nat on lo ed he ade oma to co ss st of cl ef cells and conf r ned the norm al ty of the left infer or para hyro d

The postoperat e course as neo pl at d Seru i calc um level fell to 9 8—10 0 n 100 r l and re al calc um excret o de crea ed to an a erage of 92 g/24 l urs n ge eral ard d et Seru i norgan c phos pha e as 3 0—4 6 n /100 n l a d the % TRI rose to a h gl normal value of 88—90 % Four i on hs after the opera o i the ru calc u and ph sphate values were s ll r orm al

O her exa na ons blood count serum elec r lytes and urine analys s were n r al Seru a d ur ne magr es u i valu s are h n f 1 End ger ous crea n ne learance was 110—130 ml/m n max u i u iary pec f gra ty after flu d re r t o i for l urs as 1 030 and 1 032 bef re and f r pe a o re pect vely Serum pr n ra o i and paper electroph esis

were normal Alkaline phosphatase was 18 3 falling to 12 3 K.A units after operation, enzyme electrophoresis showed normal distribution of enzyme fractions corresponding to bone and liver Mantoux test with one TU was positive

Discussion

The series of events that led to operation for parathyroid adenoma in this patient began with the accidental discovery of sarcoidosis in an asymptomatic stage with hilar and mediastinal lymphadenopathy as the only manifestation recognized A routine check of calcium metabolism as part of the verification of sarcoidosis, then revealed asymptomatic hypercalcaemia and hypercalcuria, which for reasons discussed below was considered to be due to hyperparathyroidism rather than sarcoidosis Subsequently, removal of a parathyroid adenoma eliminated hypercalcaemia and hypercalcuria before the appearance of renal damage such as would inevitably have occurred because of the very high urinary calcium excretion The urinary concentrating capacity was actually remarkably good in view of the deleterious effect of sustained hypercalcaemia and hypercalcuria on the renal concentrating mechanism (16 38) This suggests that the hyperparathyroidism as well as the sarcoidosis was of recent origin

Although all the usual biochemical criteria for the diagnosis of primary hyperparathyroidism were present in this patient, hypercalcaemic sarcoidosis was the favoured diagnosis when he was referred to us This condition, which for unknown reasons develops in only a minority of patients with sarcoidosis, is characterized by an excessive sensitivity to physiologic amounts of vitamin D (2, 3, 23, 24), which produces hypercalcaemia because of hyperabsorption of calcium from the gut (2, 3, 24) and increased bone resorption (23, 24, 27), while hypercalcuria is due partly to hypercalcaemia and partly to decreased tubular reabsorption, i e increased calcium clearance in the kidney (7) Calcium balance may be positive (2, 3) but is more often negative (23, 24, 27) The serum inorganic phosphate level is usually normal or elevated in hypercalcaemic sarcoidosis (10, 29), but several cases with hypophosphataemia have been reported (14) and in patients with normocalcaemic sarcoidosis low serum phosphorus values appear to be abnormally frequent (31) Renal phosphate leak, i e low % TRP, may be present in sarcoidosis (8) and since the clinical course can be dominated by nephrolithiasis nephrocalcinosis, renal insufficiency, polyuria and gastrointestinal complaints (33), it is evident that all the characteristic biochemical and clinical features of hyperparathyroidism may be simulated by this condition

The observation that glucocorticoids eliminate the hypercalcaemia and hypercalcuria of sarcoidosis while the hypercalcaemia of hyperparathyroidism remains unchanged led to the cortisone differential test (11) which is crucial in differentiating between these two conditions Although administration of glucocorticoid has failed in rare instances to eliminate hypercalcaemia in sarcoidosis (12 24) and has resulted in normocalcaemia in a few cases of proven

hyperparathyroidism (4, 17, 18, 26), this test remains the most reliable differential diagnostic tool available, and the decision to operate in our case was primarily due to the unequivocal negative result of the prolonged trial with prednisone

Other features, depicted in fig 1, add strength to the diagnosis of hyperparathyroidism but are of minor significance compared with the result of the steroid suppression test

Although of limited aid in hypercalcaemic states (36), the demonstration of subnormal tubular reabsorption of phosphate is considered of some value in the diagnosis of hyperparathyroidism, when renal function is normal (8) Clearly the return of the % TRP to high normal values after operation in our patient proves that the renal phosphate leak present pre-operatively was due to hyperparathyroidism During prednisone administration a small rise in the serum inorganic phosphate level occurred whereas a marked decrease in serum phosphate has repeatedly been observed in patients with sarcoidosis when treated with steroids (3 33)

Present knowledge of magnesium metabolism in hypercalcaemic sarcoidosis seems to indicate that magnesium balance in this condition is negative due to decreased absorption from the gut while urinary excretion is normal (23 25) In our patient the high urinary magnesium excretion was eliminated after removal of the parathyroid adenoma the postoperative excretion being low whereas the serum level was normal throughout This is compatible with the frequent finding that the magnesium balance is negative in hyperparathyroidism and becomes positive after parathyroidectomy (20) A likely explanation for this is illustrated in fig 1, where the daily urinary excretions of calcium and magnesium are seen to parallel each other closely in magnitude both before and after operation This accords with the common renal pathway postulated for these two divalent cations, according to which the renal tubule, when presented with an increased filtered load of one of them (in this case calcium) fails to reabsorb the other also (1)

The question of causal relationship or fortuitous association, which arises whenever two different diseases coexist, is particularly interesting in the case of sarcoidosis and hyperparathyroidism, since both diseases are of unknown aetiology The suggestion of Dent that this association occurs more often than can be accounted for by chance (12, 13) receives some support from the fact that the patient described herein is the third case of concurrent sarcoidosis and hyperparathyroidism to be reported from this country where hypercalcaemia is found in less than four per cent of patients with sarcoidosis (30) The study of this patient provides no information, however, about the nature of this association since both diseases developed simultaneously and probably recently Secretion of a parathyrotrophic factor leading to hyperplasia and later to adenoma in patients with sarcoidosis has been suggested (19) but the parathyroid hyperplasia reported in patients with sarcoidosis is probably due to simultaneous renal insufficiency (29, 37) In this connection, it is rather interesting that patients with

idiopathic hypoparathyroidism of long duration have developed sarcoidosis with ensuing amelioration of the biochemical abnormalities of hypoparathyroidism (5, 28), and one of these patients even exhibited hypercalcaemia at times (5). Whilst this adds to the complexity of parathyroid function in hypercalcaemic sarcoidosis it also proves that parathyroid hormone is not needed for its development, and the finding of normal parathyroids, adenoma, hyperplasia and atrophy in patients with hypercalcaemic sarcoidosis clearly preclude any general relationship between this condition and parathyroid function. The necessity of considering hyperparathyroidism in every case of hypercalcaemic sarcoidosis is evident, however, and a glucocorticoid differential test should be performed routinely to exclude this possibility.

Summary

The accidental X-ray finding of hilar adenopathy in a 17-year-old youth led to the diagnosis of sarcoidosis and then of hypercalcaemia and marked hypercalcuria. Steroid unresponsiveness and other laboratory evidence led to the conclusion, proved to be correct after operation, that hypercalcaemia and hypercalcuria were ascribable to hyperparathyroidism.

The clinical and biochemical similarity that may exist between this condition and hypercalcaemic sarcoidosis is discussed and the necessity of performing a glucocorticoid differential test in every case of sarcoidosis complicated by hypercalcaemia is stressed.

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Release of Glycerol and Free Fatty Acids in Human Adipose Tissue in Vitro

Effect of the specimen size

By

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The majority of investigations concerning metabolism in vitro of adipose tissue are carried out on the thinnest part of the fat pads from rat epididymis which locally consists of a layer of a few fat cells This is of importance since the thickness of the specimen is fundamental for metabolic studies in vitro (17) Vaughan (14), for instance, found differences in the rate of incorporation of isotopically labelled fatty acids into triglycerides per unit weight of rat adipose tissue when specimens of different thicknesses were used The size of the specimen is also of importance for lipolysis in rat adipose tissue The rate of release of free fatty acids from rat adipose tissue specimens has been found to be higher the higher the weight of the specimen (13)

In human adipose tissue studied in vitro, it has been established (6) that the specimen size is of importance for oxidation of, and lipogenesis from, glucose 1 ^{14}C

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Release of glycerol and free fatty acids from minced specimens of human adipose tissue has been reported to be heavier than from larger specimens (3) This phenomenon was ascribed to the larger surface per unit weight of the small specimens

The following investigation has been undertaken in order to obtain a rational basis for the preparation of specimens from human adipose tissue in future studies The effect of the specimen size upon diffusion (12), oxidation and lipogenesis (3) has been studied in earlier papers This paper describes the studied effect of the specimen size for the release of fatty acids and glycerol from human subcutaneous adipose tissue in basal state, as well as after norepinephrine stimulation This stimulation was produced for two reasons Firstly, each specimen size can be studied at two different release rates and if two specimens of different sizes produce the same release of glycerol and free fatty acid in

the basal state and show the same response to stimulation, it might be assumed that the specimens within this weight interval can be used for in vitro studies Secondly, ratios from in vivo studies of the turnover rates of free fatty acids to glycerol (1, 8) can be compared with the ratio of released free fatty acids to glycerol in vitro from such well defined specimens particularly in view of the fact that the ratios in vivo and in vitro are regulated by the same mechanisms

Material and methods

Subcutaneous adipose tissue specimens were excised from randomly selected patients operated upon for cholelithiasis Each specimen was taken at the beginning of the operation and dispensed into 20 ml Krebs Ringer bicarbonate buffer of room temperature From the large specimen were then prepared specimens weighing 400 ± 40 mg 100 ± 10 mg and 20 ± 5 mg as well as needle biopsy specimens according to a method described elsewhere (10 The inner diameter of the needle used at the aspirations was 0 8 mm All the specimens were kept in the buffer until weighed The specimen was then weighed and in quantities of 100—400 mg immersed in 3 ml Krebs Ringer bicarbonate buffer pH 7 4 containing 10 mM glucose and 5 % albumin (Bovine albumin powder Armour) The time between the excision and the beginning of the incubation was about 20—30 min

Different batches of albumin were employed but in each separate experiment the same batch was always used The incubations run in duplicate were performed during 150 min in a shaking apparatus at 37 C operating at the rate of 90 cycles/min

Norepinephrine (ASTRA) was added after 30 min. of incubation and at a final concentration of 5 μg/ml medium

Thirty and 150 min after the beginning of incubation 1 0 ml samples of the incubation medium from each incubation vessel were carried over to a test tube from which aliquots were taken for determination of the release of free fatty acids and glycerol to the medium

For determination of the accumulation of free fatty acids and glycerol in the specimens during the incubation, two biopsy specimens were extracted as controls at the beginning and two at the end of the incubation Control experiments indicated that the accumulation of free fatty acids and glycerol in the specimens were negligible during the first 30 min of the incubation (figs 3 and 4)

The free fatty acids in the specimens and medium were extracted in chloroform — methanol (2 1, v/v) and dialysed against 0 05 M acetic acid according to a modified Folch procedure (3) The subsequent analysis of the fatty acids was performed by the method described by Duncombe (6) The amount of glycerol contained in the specimens was determined in the supernatant of the dialysed free fatty acid extract by the method developed by Lambert and Neish (9) which method was also used when the glycerol concentration of the incubation medium without any previous extraction was performed Statistical analyses were carried out according to Kemp (8)

Results

Time activity curves

The relationships between the release of free fatty acids and glycerol to the medium and the time of incubation for the various sizes of specimens studied seem to be approximately rectilinear within the interval from 30 to 180 min (figs 1 and 2) The relationship between the free fatty acid and glycerol content of the specimens and the time of incubation is demonstrated in figs 3 and

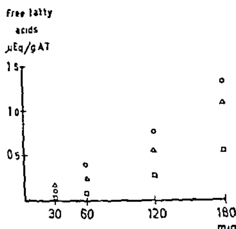


Fig. 1

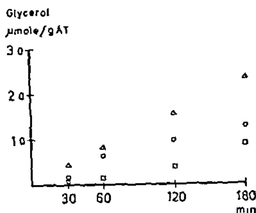


Fig 2

Figs. 1 and 2 The relationship between the time of incubation and the release of free fatty acids and glycerol respectively to the incubation medium in specimens of different weights from human subcutaneous tissue □ = 400 mg n = 3 Δ = 100 mg n = 7 o = 20 mg n = 7

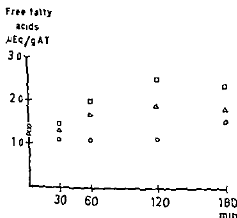


Fig 3

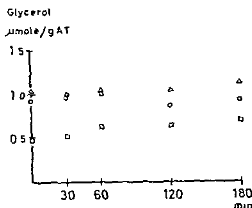


Fig 4

Figs 3 and 4 The relationship between the time of incubation and contents of free fatty acids and glycerol respectively, in human subcutaneous adipose tissue specimens of different weights. □ = 400 mg n = 3 Δ = 100 mg n = 7 o = 20 mg n = 7

4 The contents of glycerol in the specimens seem to be constant for specimens weighing 20 and 100 mg and the minor fluctuations may possibly be ascribed to differences in the preparations For the specimens weighing 400 mg there is probably an increase of glycerol content The free fatty acids seem to accumulate in all three specimens used (fig 3) On the basis of these observations, taking into account also the low specificity of the glycerol method, it was assumed that the release of glycerol to the medium and the sum of free fatty acids released to

TABLE I Glycerol release to the incubation medium in human subcutaneous adipose tissue specimens of different sizes

	Specimen size				
	400 mg	100 mg	50 mg	20 mg	Needle
Glycerol release µM/g A.T/hr[1]	0 53±0 08	0 79±0 08	0 97±0 11	1 06±0 15	0 33±0 07
n	6	11	5	11	6

Mean ± S E M
[1] A T = adipose tissue

the medium and accumulated in the specimen represented the total production of free fatty acids and glycerol from the specimens of 20 and 100 mg weight

I *Effects of specimen size*

Table I summarizes data from experiments in which release of glycerol to the medium from different sizes of biopsy specimens has been measured

The glycerol release from the 20 mg specimens was statistically higher than from the 400 mg needle specimens ($p<0\,025$ and $p<0\,005$ respectively) No difference prevailed between the glycerol release in the groups with 100 mg, 50 mg and 20 mg specimens The lowest glycerol release was obtained with the needle specimens group statistically lower than for the 100 mg, 50 mg and 20 mg groups ($p<0\,0025$, $p<0\,0005$ and $p<0\,0005$ respectively)

II *Norepinephrine stimulated lipolysis*

The release of glycerol and free fatty acids to the medium, the increase of free fatty acids in the adipose tissue and the total increase of free fatty acids are shown in table II

Addition of norepinephrine to the medium increased the release of glycerol to the medium, and this increase was statistically significant for the 100 mg as well as for the 20 mg specimens ($p<0\,025$ for both)

The increase of free fatty acids in the medium after norepinephrine stimulation was also significant for both the 100 mg and 20 mg specimens ($p<0\,0005$ and $p<0\,0025$ respectively)

The fatty acid accumulation in the unstimulated 100 mg specimen is not statistically different from the fatty acid accumulation in the unstimulated 20 mg specimen This is also the case after *norepinephrine stimulation* Subsequent to stimulation with norepinephrine there is an increased accumulation of free fatty acid in both the 100 mg and 20 mg specimens as compared with the accumulation of free fatty acids in their unstimulated controls ($p<0\,025$ and p 0 05 respectively)

A significant increase of the release of the total free fatty acids is found for both the 100 and 20 mg specimens subsequent to norepinephrine stimulation ($p<0\,005$ for both) There was no dif

TABLE II Free fatty acid and glycerol release from subcutaneous adipose tissue specimens of different sizes with and without norepinephrine stimulation

Specimen size	FFA release to medium μEq/g A.T.¹/hr	FFA accumulation in μEq/g A.T.¹/hr	Total FFA increase μEq/g A.T.¹/hr	Glycerol release μM/g A.T.¹/hr	FFA / Glycerol
100 mg n = 12	1.03±0.19	0.64±0.16	1.67±0.27	0.79±0.09	2.20±0.30
100 mg+NE n=12	1.74±0.23	1.39±0.30	3.13±0.34	0.99±0.14	3.57±0.52
20 mg n=12	1.55±0.34	0.47±0.12	2.02±0.36	0.88±0.10	2.23±0.28
20 mg+NE	2.27±0.40	0.87±0.18	3.14±0.41	1.15±0.17	2.91±0.13

Mean ± S.E.M.

¹ A.T. = adipose tissue

ference between the total release of free fatty acids from 100 mg and 20 mg specimens in the basal state and this was also the case when the corresponding comparison was made for the norepinephrine stimulated specimens.

The ratio of total fatty acid to glycerol production showed no difference between specimen size, basal or stimulated, but increased after norepinephrine stimulation for both the 100 mg and 20 mg specimens ($p<0.05$ and $p<0.005$ respectively).

Discussion

The constant concentration of glycerol in adipose tissue specimens in vitro, found in the present investigation, was also reported by Östman (17) after a short initial period of decrease. This decrease was explained as a diffusion of glycerol, which had accumulated during the excision from the specimen to the incubation medium. The decrease was not traced in the present investigation, but the difference between the results can be explained by the fact that the specimens used in the present study were kept in buffer until prepared into small specimens which were also kept in buffer until weighed, thus permitting accumulated glycerol to diffuse from the specimens to the medium before the analyses. In addition, the efflux of small molecular substances, such as sucrose from human adipose tissue specimens weighing between 20 and 100 mg has been found to be very rapid (11).

A low release of glycerol and free fatty acids to the medium was obtained with the needle biopsy specimens and, possibly due to traumatization during the preparation, they have been found less active in other respects as well than conventionally prepared specimens (5). Also the largest specimens weighing 400 mg gave a low release of glycerol to the medium, which possibly can be ascribed to a delayed diffusion between the medium and these large specimens. Due

to the low glycerol release from these two specimen types, they were not used in further studies

The release of glycerol from the adipose tissue was found to be of the same rate in the unstimulated specimens of both 20 and 100 mg weight, and no difference between these sizes is found after norepinephrine stimulation The same results were obtained when the total release of free fatty acids was studied under identical conditions It is therefore likely that specimens in the range of 100 and 20 mg weight are most appropriate for lipolytic studies of human adipose tissue in vitro when utilizing specimens obtained by cutting

In the rat epididymal fat pad, incubated in vitro, free fatty acid release in relation to the glycerol release can be utilized as a measure of reesterification in a net balance method (14, 15) Whether it is possible to calculate the reesterification rate in human adipose tissue on the basis of the release of free fatty acids and glycerol is not demonstrated and the method may be objected to on account of the concentration of di and monoglycerides not being known in human adipose tissue our knowledge of the reesterification rate of partial glycerides in human fat is also incomplete Furthermore, glycerol is metabolised in human adipose tissue only to a minor degree (4) but it is not known to what extent glycerol is derived from sources other than glycerides

In connection with studies on the influence of the sympathetic nervous system on mobilization of free fatty acids and glycerol from human adipose tissue it has been shown that the ratio of the turnover rate of free fatty acids to the turnover rate of the glycerol may exceed 3 1 during rest, exercise and recovery from exercise, and reach values up to 7 1, but such high ratios were not found in the present investigation (1, 7) Ratios of this magnitude might imply incomplete hydrolysis and accumulation in the adipose tissue of diglycerides and monoglycerides, or a rapid synthesis of fatty acids

The ratio of the total release of free fatty acid to the release of glycerol (cf table II) in the present investigation may be above three subsequent to norepinephrine stimulation, these findings are principally in accordance with the results from studies of the turnover rates of free fatty acids and glycerol in vivo and suggest further studies, inter alia, of the content of partial glycerides of human adipose tissue under different conditions

Summary

The release of free fatty acids and glycerol from human subcutaneous adipose tissue was investigated in specimens of different sizes in basal and norepinephrine stimulated states The glycerol release was found to be of equal magnitude for specimens weighing between 20 and 100 mg but lower for specimens weighing 400 mg and needle biopsy specimens The total release of free fatty acids was found to be of equal magnitude for 20 and 100 mg specimens Glycerol as well as free fatty acid release was increased after norepinephrine stimulation, the increase being of the same magnitude for specimens of 20 and 100 mg weight

These results suggest optimal conditions of in vitro studies of lipolysis for specimens in the weight range of 20—100 mg

A release of free fatty acids exceeding glycerol release by a factor of more than three subsequent to norepinephrine stimulation of human adipose tissue suggests accumulation of mono- and diglycerides in the adipose tissue

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On the Composition of Human Adipose Tissue

By

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During studies in vitro by the incubation technique on human adipose tissue it has been demonstrated that the incubated specimens increase their weight by water uptake (21, 26) When the composition of human adipose tissue is to be analysed this phenomenon will raise the question whether different ways of handling the specimens prior to analysis have an influence on the result and whether the divergence concerning the water contents of human adipose tissue reported in the literature (1, 8 14, 15, 16, 17, 24, 25, 29) can be explained by this circumstance

The total water content of adipose tissue specimens is almost equal to the sodium space, estimated by direct analysis of adipose tissue (24) The size of the extracellular space in human adipose tissue has also been investigated by in vitro methods for other substances, such as sorbitol, raffinose and sucrose (7, 21) In determining the size of the extracellular space in vitro the time required for equilibration between the incubation medium and the extracellular space is of importance for estimating the usability of the specimens for metabolic studies, since the time required for reaching equilibrium is a criterion of the diffusion property of the specimen

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The object of the present investigation has been to study variations of the water contents under different in vitro conditions, and to study the composition of the adipose tissue. In connection with this the relationship between different components of the adipose tissue has been studied for discussion of suitable reference substances relative to experiments in vitro In order to obtain information of specimen sizes suitable for metabolic studies in vitro, the equilibration between the incubation medium and the specimen was studied with regard to outflow from as well as inflow to the specimen in the case of different substances and with specimens of various sizes

Material and methods

A Composition of adipose tissue

In connection with operations for chole lithiasis a piece of subcutaneous adipose tissue was excised from patients not suffering

from extreme obesity (weight index less than 13) at the beginning of the operation for analysis of the adipose tissue and immediately transferred either to a sealed plastic vessel moistened with 1 ml Krebs-Ringer bicarbonate buffer (23 cases, 17 women and six men) or, in order to compare the effect of the amount of buffer in the storage vessel, to a sealed plastic vessel containing about 30 ml bicarbonate buffer, allowing the specimen to float (15 cases, seven men and eight women) The specimens were dissected into smaller pieces, macroscopically free from blood and connective tissue, from the central part of the specimens The amount of analysed tissue was about 100—400 mg The preparation was carried out in a moistened chamber

Dry weight determinations were performed by placing dissected pieces into small glass-stoppered vials and drying them at 50 C to constant weight in an evacuated desiccator containing phosphorus pentoxide The lipids were extracted in ethanol ether (2 1 v/v) at 50° C for one hour from specimens which, immediately after weighing, were homogenized in a Potter Elvehjem all glass homogenizer

This extraction method has been compared with extraction in methanol chloroform 1 2 v/v) without homogenization and the results for both methods are practically identical

The lipid extract was taken to near dryness reextracted with methanol chloroform and dialysed against physiological saline After dialysing over night, the supernatant was removed by suction and the infranatant was diluted to volume Aliquots for determination of total lipids and the various lipid fractions were taken from this extract

Determination of total lipids was carried out by taking aliquots of the lipid extracts to dryness on a boiling water bath and then drying to constant weight Triglycerides were determined from the amount of glyceride glycerol ascertained according to the method developed by Carlson and Wadström (10) as modified by Carlson (9), assuming the mean molecular weight of the fatty acids of the triglycerides to be 282 (4) Cholesterol was determined after saponification according to the method of Sperry and Webb (27) Phospholipids were determined by wet combustion according to the method described by Svanborg and Svennerholm (28) Phospholipids were calculated from the lipid phosphorus by multiplying by a factor of 25

Determination of protein The delipidised fatty tissue was hydrolysed with sodium hydroxide, and aliquots from the hydrolysate were taken for determination of nitrogen according to Lowry et al (23) A standard curve for nitrogen was drawn up, based on nitrogen determination in pooled delipidised adipose tissue by means of a micro Kjeldahl method according to Clark (11)

Deoxyribonucleic acid (DNA) was determined according to Webb and Levy (32)

B Studies of the extracellular space

The preparation of the subcutaneous adipose tissue excised under the same conditions as above and transported floating in buffer, was performed with a pair of scissors and forceps or by aspiration of needle biopsy specimens The incubations were performed in a medium consisting of Krebs-Ringer bicarbonate buffer, containing glucose and albumin (Bovine albumin powder, fraction V Armour) at a final concentration of 10 mM and 5 %, respectively For measuring the volume of the extracellular space, a substance in the sequel called indicator was added Inulin, sucrose U ^{14}C and ^{131}J albumin were used as indicators The incubations were carried out in a modified Warburg apparatus, making 90 cycles per minute, at 37° C and pH 7 4

The diffusion was investigated by two different methods

I Diffusion of indicator from the specimens (Efflux technique)

Specimens weighing 100 mg or 20 mg were preincubated for one hour in 2 ml of a

medium containing sucrose U ^{14}C (CFB 4, The Radiochemical Centre Amersham) in a total activity of 8×10^{5} cpm or radio iodinated human serum albumin (RISA, The Radiochemical Centre, Amersham) corresponding to a total activity of about 10^{6} cpm and with a single specimen in each vessel Prior to use RISA was dialysed against physiological saline in order to remove free iodine Subsequent to preincubation the specimen was first dipped for five seconds in a vessel containing two ml of medium free from radioactivity, and then, at different intervals as shown in fig ?, in a similar buffer The eluted carbon radioactivity in each of these vessels was determined by measuring the radioactivity in an aliquot of the eluate in a liquid scintillation spectrometer, as will be described in the next section, and the iodine radioactivity in a Tracerlab gamma ray spectrometer

II *Diffusion of indicator from the medium into the specimens (Influx technique)*

Inulin Specimens weighing about 200 mg were incubated in the abovementioned medium containing inulin, 2 mg per ml (Inulin, puriss Laevosan Gesellschaft) during various intervals The specimens were then rinsed in 10 ml physiological saline for 10 seconds and blotted cautiously on a moistened filter paper They were then transferred to glass stoppered tubes containing 2 ml redistilled water cut into small pieces and soaked for 15 min on a boiling water bath Inulin was analysed according to Hubbard and Loomis (19) Needle specimens were incubated in a similar way, but after incubation these specimens were transferred to a stainless net in a funnel connected to a water suction pump, rinsed with 3 ml physiological saline transferred to tubes and analysed for inulin as above

Sucrose U ^{14}C Specimens weighing 100 mg and 20 mg were incubated in a 2 ml medium containing sucrose U ^{14}C in a total activity of 4×10^{4} cpm and with 100 mg adipose tissue in each incubation vessel After incubation the various biopsy specimens were dipped in physiological saline for 10 seconds and dried with a moistened filter paper They were then soaked in 2 ml redistilled water on a boiling water bath for 5 min in glass stoppered tubes and left for two hours, after which the content was filtered A quantity of 0 5 ml filtrate was taken to dryness in glass vials and dissolved in 1 0 ml Hyamine 10X. Ten ml scintillation liquid (4 g of 2 5 diphenyloxazole and 0 1 g of 1 4 bis-2 (4 methyl 5 phenyloxazolyl) benzene in 1,000 ml of toluene) was added and the radioactivity registered in a Packard Tri Carb liquid scintillation spectrometer Quenching of ^{14}C was corrected for by use of an internal standard When calculating the extracellular space the amount of indicator in the specimen was converted to the corresponding volume of the incubation medium and expressed in percentage of the specimen weight The result obtained after an incubation period of one hour by influx technique was selected for comparison of the extracellular space of the different indicators

Results

Table I shows the values of the composition of adipose tissue in respect of total lipids, triglycerides, cholesterol, phospholipids, nitrogen and DNA When the adipose tissue was kept in a moistened chamber, the water content was 8 9 %, as shown in table I, against 22 4 % when the specimens were kept floating in buffer In order to study this difference, the weight of specimens weighing 100 mg and 400 mg respectively, was registered at varying intervals during incubation The results are shown in fig 1 As may be seen, there was an increase of the wet weight during the incubation of both specimen sizes for 30 min, after which the weight was constant Similar results were also obtained with 20 mg specimens

TABLE I The composition of specimens from human subcutaneous adipose tissue All figures are calculated as mg per 100 mg wet adipose tissue

	Dry weight	Total lipids	Triglyceride	Cholesterol	Phospholipids	Nitrogen	DNA
Mean ± S E M	91 1±0 63	90 3±0 92	86 0±1 67	0 16±0 01	0 15±0 01	0 26±0 02	0 0074±0 0010
n	23	23	12	23	23	23	11

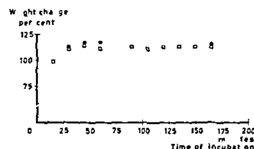


Fig 1 Weight increase in subcutaneous adipose tissue specimens of different sizes from one patient • and □ indicate specimens of 400 mg and 100 mg weight respectively The specimens were incubated in Krebs Ringer bicarbonate buffer containing albumin and glucose at a final concentration of 5 % and 180 mg % respectively at pH 7 4 and 37° C The specimens were weighed every 15 min The incubations were run in duplicate The initial weight was defined as 100 %

The relationship between different components of adipose tissue is shown in table II in the form of equations of the regression lines and correlation coefficients As may be noted there is a negative relationship between the structural components such as nitrogen and DNA on the one hand and neutral lipids on the other Among the structural components, nitrogen was found to be correlated to phospholipids as well as to DNA

When the extracellular space was studied, controls were performed by a technique described elsewhere (7) which showed that no incorporation of ^{14}C from sucrose ^{14}C into lipids and carbon dioxide took place in adipose tissue

TABLE II Statistical analysis of the relationships between the content of various components in human subcutaneous adipose tissue

Groups compared	n	Equation of regression line	Correlation coefficient	P
Nitrogen — dry weight	23	y=—0 022x +2 26	—0 72	<0 005
Phospholipids — dry weight	23	y=—0 0073x +0 82	—0 81	<0 0005
DNA — total lipids	11	y=—0 42x +39 4	—0 86	<0 0005
Phospholipids — nitrogen	23	y= 2 58x —0 13	0 75	<0 0005
DNA — nitrogen	11	y= 0 0039x +0 17	0 81	<0 0025
Cholesterol — phospholipds	23	y= 0 19x +0 17	0 37	<0 05

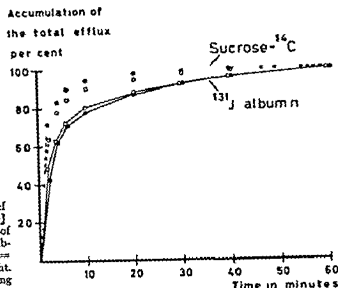


Fig 2 The time lapse of the efflux of sucrose-U ^{14}C and ^{131}J albumin from biopsy specimens of different sizes from human subcutaneous tissue o—o—o— = specimens of 100 mg weight. •—•—• = specimens of 20 mg weight. n = 4

The results of the experiments with the efflux technique are shown in fig 2 The efflux of the indicators was independent of the specimen size Within 10 min 90 % of the total elutable sucrose had passed out from the specimen as against 25 min for the same amount of albumin The remaining activity of ^{131}J albumin in the specimen was determined after the last elution and found to be less than 10 % of the total eluted activity

The course of the influx when inulin and sucrose U ^{14}C and different types of biopsy specimens were used is shown in figs 3 and 4 With inulin equilibration was reached within 10 min for the needle specimens whereas it appeared to be achieved more slowly in respect of larger specimens When sucrose was used equilibrium seemed to be reached for the 20 mg specimens in 10—20 min, where after only a small increase was registered whilst the 100 mg pieces seemed to reach a plateau somewhat later at about 30 min

The volume of the extracellular space determined by means of inulin and sucrose using the influx technique is shown in table III When inulin is used

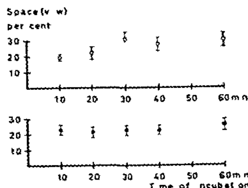


Fig 3 Inulin space in different types of human subcutaneous adipose tissue preparations after different periods of incubation o and • = specimens of 200 mg weight and needle specimens respectively Number of investigations see table III Mean ± S E M

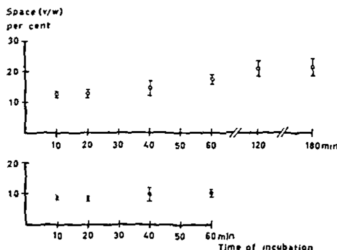


Fig 4 Sucrose space in different sizes of human subcutaneous adipose tissue preparations after different periods of incubation o and • = specimens of 100 mg and 20 mg weight respectively Number of investigations see table III Mean ± S E M

TABLE III Inulin and sucrose spaces in human subcutaneous adipose tissue specimens of different sizes Mean ± S E M

Indicator	Specimen size	Space % (v/w)
Inulin	200 mg n 7	17 7 ± 1 34
	Needle n 5	10 4 ± 1 01
Sucrose	100 mg n = 7	30 5 ± 4 73
	20 mg n = 7	25 7 ± 3 49

as indicator the volume of the extracellular space is lower for the needle biopsy specimens than for the larger specimens As far as the two sizes of specimens used in the investigation of sucrose were concerned, no difference could be observed

Discussion

The reason for the weight increase, which was also noted by Kahlenberg and Kalant (21), is partly the refilling of spaces emptied during the preparation when the specimen seems to be compressed The rapid collection of blood on the underside of the specimen after excision indicates a rapid emptying of blood vessels Since the buffer used is isotonic with serum, water uptake as a result of the osmotic changes during the experiments is unlikely In the present study, weight increases have been found both with and without albumin in the medium, and this tends to indicate a lack of albumin effect (cf 26)

The water content of human adipose tissue has been investigated by numerous authors (e g 1, 8, 14, 15, 16, 17, 24, 25, 29), and the results vary considerably A possible explanation is that the results are influenced by the water soaking during the storage in buffer, the times of which have varied in different investigations It has recently been shown that the extracellular space is related to the size of

adipose tissue cells, and it seems possible that the varying structure of adipose tissue is also of importance (5)

The relationship between the number of fat cells and the content of DNA in adipose tissue has been demonstrated by Bjorntorp and Martinsson (6) Analyses of different parameters show that there is an inverse relationship between, say, DNA and total lipids The variation of the water contents and the above mentioned relationship between DNA and the total lipids thus disfavours the use of wet weight as reference basis The contents of cholesterol, phospholipids and nitrogen are in close agreement with earlier studies (2 14, 18, 20, 30, 31)

In the present investigation positive correlations were found between DNA and nitrogen and also between nitrogen and phospholipids Nitrogen and phospholipids therefore, ought to be a complement to DNA as a reference substance in adipose tissue in in vitro experiments Nitrogen in adipose tissue after in vitro incubation of the specimen is often difficult to determine since most incubations are performed in an albumin containing medium involving a risk of contamination from the medium when analysing the nitrogen content of the specimen.

When the equilibration between the medium and the extracellular space was studied, the elution of sucrose was found to be somewhat more rapid than for albumin. This may be explained by the fact that the two substances are distributed in two different compartments and that the elution from these compartments (21) proceeds at different velocities Another possibility is that the two substances have different diffusion constants, which would influence the diffusion rate The initial phase of the elution proceeds very rapidly and this tallies with the results of investigations on fat pads from rats (12, 21) The efflux method seems to be more convenient than the influx technique when the initial rapid phase of the equilibration is to be studied, possibly due to the use of the same specimen throughout the investigation Kahlenberg and Kalant (21) established equilibrium between the medium and the extracellular space of human adipose tissue within 10—20 min when sorbitol was used as indicator, which agrees very well with the results of the present investigation The equilibration seems to proceed rapidly enough to permit the use of biopsy specimens of 20—100 mg weight for metabolic studies in vitro of human adipose tissue If the results from similar investigations of specimens from other tissue used for in vitro studies are compared Kipnis and Cori (22) using intact rat muscle diaphragm and thiosulphate as indicator found equilibrium to be established after five minutes, but with a cut diaphragm the equilibration curve levelled off after 30 min, but no definitive equilibration was reached after three hours which was assumed to be an effect of the damage caused during preparation

When the needle specimens were used in the present study a smaller size of extracellular space was observed in comparison with the other specimens This can partly be ascribed to the different handling of these specimens which may cause a loss of indicator resulting in a low extracellular space value and there-

fore the values in respect of the large samples are probably more correct

The size of the extracellular space found in human subcutaneous specimens in the present study is higher than the 15 % reported by Morse and Soeldner (24) and obtained by using sodium as extracellular space indicator in subcutaneous adipose specimens Kahlenberg and Kalant (21) found an extracellular space of 16 6 % in omental human adipose tissue The results of the present study agree more closely with the findings of an extracellular space of about 20 % as reported by Bjurulf (3) and Björntorp et al (5) The difference between the extracellular space of inulin and sucrose may possibly be due to a distribution of the indicator in different spaces and also to osmotic differences

Summary

The composition of human subcutaneous adipose tissue has been investigated with regard to dry weight, total lipids, triglycerides, phospholipids, cholesterol, nitrogen and deoxyribonucleic acid The water content was found to increase when the specimens were stored in buffer This might explain some of the differences between earlier investigations of human adipose tissue composition.

As a reference substance deoxyribonucleic acid can be replaced by phospolipids and nitrogen, provided that there is no risk of contamination of proteins from other sources than adipose tissue The use of total lipids as a reference substance is inadvisable as the total lipids and the amount of cells expressed as deoxyribonucleic acid are negatively correlated

The size of the extracellular space was investigated by means of sucrose and inulin. The rate of equilibration measured as outward diffusion from the extracellular space of the specimen into the incubation medium was established by means of sucrose and albumin. The efflux seems to be more rapid for sucrose than for albumin and various possible reasons for this are discussed

The equilibration between the specimens of 20—100 mg weight and the incubation medium proceeded rapidly enough to permit the use of specimens of these sizes for in vitro studies

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From the Hospital for Infectious Diseases and the Central Bacteriological Laboratory, Stockholm, Sweden

Infection with Bacteria, Mycoplasma, and Viruses in Acute Respiratory Illness

By

GORAN STERNER, ARNE SVEDMYR, GOSTA TUNEVALL, MARIANNE FORSGREN, HANS FRANSEN, SIGVARD WOLONTIS and P O GRIBBE

Since 1963, after orientating studies (10, 11, 16), we have at the Hospital for Infectious Diseases subjected patients with acute respiratory illness to a standard procedure of examination (clinical, bacteriologic and serologic) in order to elucidate the aetiologic factors likely to take part in their pathologic conditions This report deals with the initial part of this study, from January to middle of May 1963, when the investigation was temporarily interrupted by a smallpox epidemic

Other studies on acute respiratory illness have been reviewed in our previous reports (10 11, 16) A few recent investigations only will be mentioned here

Urquhart et al (17) found in hospitalized children that, with the exception of the possible association between upper respiratory tract infection and adeno viruses, no specific virus or virus group was associated with any particular disease category Bacteria were often isolated but no serologic tests were performed in order to elucidate their significance The complexity of the infections was stressed Portnoy et al (9) in a similar material found serologic responses to more than one viral agent in 13—14 % whether respiratory illness was present or not Cross-reactions between related agents, one virus activating another or provoking so-called anamnestic reactions, were discussed but simultaneous or sequential virus infections probably acquired prior to hospital admission were accepted as a common cause of multiple serologic reactions No bacteriologic study was carried out In a combined virologic and bacteriologic study on infants and small children with acute respiratory illness Wigand et al (19, 20) found serologic reactions against viruses in 54 % Viral mixed infection was found in about 10 % Among 96 children 74 serologic reactions against bacteria were found some cases dis

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TABLE I Age distribution of all patients with acute respiratory illness and of those investigated Pneumonic cases separated from those without pneumonia

Age (yrs)	Cases without pneumonia		Cases with pneumonia		Total	
	All cases	Inves tigated	All cases	Inves tigated	All cases	Inves tigated
— 6	26	2	7	2	33	4
7—15	21	6	8	6	29	12
16—29	25	13 a)	15	10	40	23
30—49	20	16 b)	25	20	45	36
50—69	35	24 b)	51	32	86	56
70—	15	15	42	30	57	45
Total	142	76	148	100	290	176

Including a) two case, b) one case } without clinical signs of pneumonia but not examined with X ray of the chest

playing more than one antibacterial re action Bacterial findings and serologic reactions coincided poorly and the lat ter were thought to be more important The antibacterial responses often con sisted of titre decreases Bacterial infec tion is seen as an important factor in respiratory illness Berg et al (1) in our country have continued studies on upper respiratory illness in conscripts In this type of material they found bacterial agents to cause only a negligible fraction of acute respiratory illness

Material

In some respects the material is incomplete Patients admitted on Fridays and Saturdays could not be included for technical reasons Patients with evident or suspected scarlet fever or other streptococcal disease were excluded Patients suspected to suffer from infectious mononucleosis were investigated in a separate series

The material is presented in table I Unfortunately many children had to be excluded due to an inadequate follow up notably children without pneumonia The cases were evenly distributed over the obser vation period, with one exception. An in fluenza A epidemic increased the number of cases in March to about double the normal rate Slightly more women than men were admitted (156/134) and examined (102/74)

Methods

Material for cultures of bacteria and myco plasma and blood samples for serologic in vestigation were collected according to the schedule given in table II When cultures had to be done at other times the results of these were counted together with those of the tests scheduled

A conventional culture technique was used for bacteria and the method described by Chanock (2) for mycoplasma Bacterial find ings were recorded only when made in direct plate cultures and findings of only a few colonies were not counted

The serum samples from each patient were kept in the frozen state until all of them could be assayed together Some results of Paul Bunell s reaction and cold agglutinin tests (CA) emanate, however from single determinations

TABLE II Schedule for taking samples For patients admitted late in the afternoon the schedule was postponed for one day Depending on the week-end situation small deviations could take place after the first three days

Day no counted from admission	Throat swab	Naso-pharyngeal swab	Nasal swab	Expectorate (if produced)	Blood sample
1	B	B+MP	B	B+MP	S
2	B		B	B	
3	B	B	B	B	
7	B	B	B	B	
10					S
14	B	B	B	B	
21	(B)	(B)	(B)	(B)	S

Bacterial culture = B Culture for Mycoplasma pneumoniae = MP Serologic investigation = S

TABLE III Potentially pathogenic agents and the serologic tests used for indication of infection with same agents In a few cases anticolilysin tests (ACol) (18) were also carried out

Agent	Test
Influenza A and B viruses	Complement fixation (soluble antigen) (3 4 13)
Adenovirus	Complement fixation (group antigen) (3 4 13)
Respiratory syncytial virus	Complement fixation (3 4 13)
Parainfluenza viruses	Complement fixation (3 4 13)
Psittacosis agent	Complement fixation (4 13)
Infectious mononucleosis *agent(s)*	Paul Bunnell and Davidsohn tests
Mycoplasma pneumoniae	Complement fixation and cold agglutinin test (4 12 13)
Pneumococci	Antipneumolysin (APn) (15)
β-streptococci	Antistreptolysin (AS) (5 6 7)
Staph aureus	Antistaphylolysin (Asta) (8)
Haemophilus influenzae	Complement fixation (AHI) (14)

The serologic tests performed on each serum are enumerated in table III With very few exceptions the serum samples were sufficient for all tests listed

For the antilysin tests titre changes were considered significant when more than two-fold For complement fixation tests (CF) and for heterophile agglutination tests a four fold change was the criterion In the following table besides the significant titre increases also decreases of the same magnitude will be reported though separately from the increases Finally high but not significantly changed titres ($\geqslant 64$) against virus and mycoplasma and in the cold agglutination test ($\geqslant 8$) are reported and will be discussed

The assignment of patients to the pneumonic or non pneumonic groups was based on roentgenologic findings in all but three cases

TABLE IV Serologic reactions against viruses and Mycoplasma pneumoniae Significant titre rises (rise) titres without significant change of 64 or more — for CA eight or more (high) and titre decreases (fall)

Agent	No pneumonia			Pneumonia			Total		
	Rise	high	fall	Rise	high	fall	Rise	high	fall
Adenovirus	1			1			2		
Influenzavirus A	22	5		20	4	2	42	9	
Influenzavirus B				1			1		
Parainfluenzavirus 1—3	5	1		6	6		11	7	
Respiratory syncytial virus	1			1			2		
Psittacosis agent	2			3			5		
Mononucleosis agent(s)	1						1		
Mycoplasma pneumoniae									
CF	5	2	1	23	4		28	6	1
CA	2	1	4	19	2	6	21	3	10
CF and/or CA	6	3	4	27	2	4	33	5	8
Total	38	9	4	59	12	6	97	21	10

If there is a rise against any of the parainfluenza types the case is recorded as a rise For mycoplasma if there is a rise in one reaction the case is counted as a rise if no rise occurred but a fall the case is recorded as a fall

Results

Viruses and Mycoplasma pneumoniae (MP)

A summary of the serologic findings is given in table IV As many as 97 titre rises indicating fresh infection with virus or MP were found in 87 out of the 176 patients

Increasing high and decreasing titres against influenza A antigen were evenly distributed between pneumonic patients and non pneumonic ones, whereas out of 46 with such reactions against MP only 13 patients escaped pneumonia From nine of the 33 patients with serologic evidence of MP infection mycoplasma could be isolated, all but one isolation being made from pneumonic patients and all of them from expectorate

A difference as to the time distribution of cases with serologic reactions against these two agents is shown in fig 1, where the patients are arranged according to the day of onset of disease Influenza A culminated very sharply in March and then dominated the picture It is notable that the occurrence of high and falling titres against influenza A coincides well with that of titre rises Titre rises against MP occurred at a moderate frequency during the whole period Further, the age distribution of influenza A infection clearly differed from that of MP infection as seen in table V

The relations between the CF reactions against MP and the cold agglutinin (CA) titres are shown in table VI Out

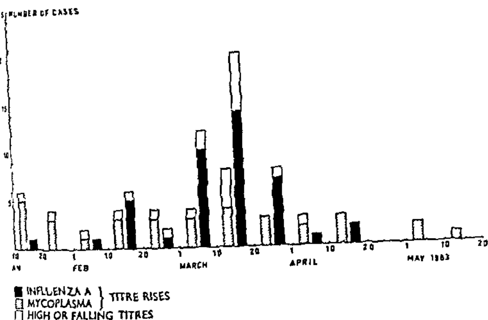


Fig. 1 Distribution in time of cases with serological reactions against influenza A virus and with Mycoplasma pneumoniae

TABLE 5 Age distribution of infections with the more common agents as indicated or suggested by serological reactions (increases and for bacteria decreases) or for Gram negatives other than H influenzae by isolation

Age group	—6	7—15	16—29	30—49	50—69	70—
No of patients	4	12	23	36	56	45
Influenza A		1		7	17	17
Mycoplasma pneumoniae	1	4	12	10	6	
Pneumococci	1	3	6	13	22	20
Staphylococci			1	2	3	2
H. influenzae	1		6	5	12	7
Other Gram-negatives		1	2	4	4	18

of 28 patients with rising CF titres 16 also presented rising CA titres (57 %) If high and falling titres in both reactions are counted the figures rise to 23 out of 35 patients (66 %) Conversely, of 21 increasing CA titres 16 were accompanied by rising CF reactions (76 %), if high and falling titres are included, out of 34 CA reactions 23 were combined with reactions in the CF system (68 %) Ten instances of falling CA titres occurred whereas one instance only of falling CF titre was observed

Bacteria

In our previous study (16) the APn re-

TABLE VI Relation between complement fixation titre against Mycoplasma pneumoniae and cold agglutinin titre

CA titre	CF titre against MP				
	Rise	High	Fall	Neg	Total
Rise	16	2	0	3	21
High	1	1	0	1	3
Fall	2	0	1	7	10
Neg	9	3	0	—	12
Total	28	6	1	11	46

actions were subjected to an analysis which indicated that titre decreases of the same magnitude as the increases considered to be significant were also indications of a recent infection with pneumococci In the present material the same treatment of the AHI values gives a similar result This is shown in fig 2, from which will be seen that the titres of patients harbouring H influenzae, whether they suffer from bronchopneumonia or not differ from those of patients not found to carry this organism A decrease of the mean AHI titre of H influenzae carriers is also observed in spite of the fairly short observation time

The occurrence of bacteria of serologic responses against the same and of instances when both a bacterium and the corresponding antibody reaction were found is given in table VII There, bacterial findings after the third day in hospital are omitted, as well as serologic reactions related to such findings A few characteristic features will be pointed out

Out of 89 bacterial findings 60 only were accompanied by serologic responses and, conversely, 45 out of 105 serologic reactions were found in the absence of the corresponding bacterium Out of those 45 reactions 32 occurred in patients given antibacterial treatment before admission Of the 105 significant titre changes as many as 36 were decreases The APn reaction contributed most to this high proportion of decreases, 32 out of 62, whereas decreases were more rare in other reactions, four out of 43

A similar report of bacterial findings emerging after the third day of hospitalization or persisting after more than five days in hospital is given in table VIII Superinfections with pneumococci were frequent in patients not given antibacterial treatment in hospital, whereas infections with H influenzae and other Gram negative rods were common in patients treated with penicillin, and fungi emerged mostly in patients treated with broad spectrum' drugs or combinations

Mixed infection

Out of the 176 patients 61 displayed titre rises against viruses, three of them against two different agents Titre rises against MP occurred in 33 cases, eight of which also developed antiviral reactions Out of 90 patients without evidence of viral or MP infection 49 had reactions against bacteria, five of them against two bacterial species In 30 of the 61 patients with evidence of viral infection antibacterial reactions also occurred, in six of them against two species Of 25 patients with serologic reactions against MP but not against virus, 13 also reacted against bacteria

Fig 2. AHI titres after different periods of illness counted from day of admission or when H influenzae emerged after third day in hospital from the day of the last negative culture

Patients without H influenzae findings, H influenzae carriers without pneumonia and carriers with pneumonia are reported separately. Averages titres of the three groups have been calculated for the periods marked in the diagram and connected to mean titre curves

Statistical treatment

Titres expressed as ^{2}logs with titre 10 as zero

All titres in non carriers	0 132 ± 1 364	(1)
Titres in pneumonic carriers day 4—25	2 732 ± 1 999	(2)
Titres in non pneumonic carriers day 4—25	2 097 ± 1 572	(3)
All H. influenzae carriers after day 25	0 579 ± 1 118	(4)

Differences 1—2 8 130 eD
1—3 7 101 eD 4 — (2 + 3) t = 4 220

Thus titres of carriers on days 4—25 differ significantly from titres of non-carriers, and among titres of carriers those recorded after the 25th day are significantly lower than those found during days 4—25

Figures
○ Titre of patient without H influenzae
◐ Non-pneumonic patient with H influenzae
● Pneumonic patient with H. influenzae

Averages

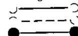


TABLE VII Isolations during the first three days after admission of bacteria *without* serologic reaction against the same *together with* such a reaction and findings of serologic reactions in the absence of corresponding bacterium. The decreases included in totals are given in brackets and serologic reactions against bacteria found after the third day are excluded. Treatment refers to antibacterial treatment prior to admission

Finding	Pnc	Pnc + APn	APn	Str	Str + AS	AS	Aur	Aur + ASta	ASta	H I	H I + AHI	AHI
Pneumonia (100)	3	24 (11)	18 (8)	1	3	1	3	2	5 (1)	3	10	4
No pneumonia (76)	6	11 (6)	9 (7)	1	3	4 (1)	5	1	2 (1)	7	6	2 (1)
Not treated (91)	8	24 (12	6 (4)	2	4	3	4		3	5	9	1
Treated (85)	1	11 5	21 11		2	2 (1)	4	3	4 (2)	5	7	5 (1)
Total (176)	9	35 17	2 15	2	6	5 (1)	8	3	7 (2)	10	16	6 (1)

In 14 cases Gram negative rods other than H I were isolated in one case meningococci and in one case Candida albicans

TABLE VIII Findings of bacteria emerging after the third day from admission or remaining more than five days after start of antibacterial treatment. Findings accompanied by serologic responses are reported separately and the observations are divided according to treatment prior to sampling

Finding	Pnc	Pnc + APn	Str	Str AS	Aur	Aur + ASta	H I	H I + AHI	Gr neg	Fungi
No treatment 63	12	5			1	1	1	4	3	1
Penicillin (76					2		7	9	16	1
Broad (37)	1			1	2				3	4
Total (176)	13	5		1	5	1	8	13	22	6

Broad includes real broad spectrum antibiotics combinations and sulpha drugs

To sum up 41 patients had no serologic reaction (23 %) 82 had reactions against one agent (47 %) 39 against two (22 %) and 14 against three (8 %)

Discussion

Our approach to the evaluation of isolations and serologic findings has been described elsewhere (16) and will not be restated here In this earlier study in

fections with potential pathogens were found in about 75 % of the material Since then the number of antigens used for the serologic investigation has been increased The cold agglutinin test has been supplemented with the CF test for MP antibody, and parainfluenza and RS viruses have been added In spite of these additions the proportion of cases with a serologic indication of recent infection in the present material is similar when same criteria are adopted, viz. 77 %

As in previous investigations the aetiological pattern was very complex Among the 176 cases, viral agents alone were concerned in 29 cases as indicated by significant titre rises, mycoplasma alone in 12, and bacteria alone in 49 cases Serologic reactions against agents from more than one of these groups occurred in 45 cases The complexity is still more pronounced if antibacterial reactions emerging during the stay in hospital are counted

Among the agents studied the following dominate influenza A virus (present in 42 cases as judged by titre rises) Mycoplasma pneumoniae (33 cases) pneumococci (62 cases) and H influenzae (22 cases) The following comments deal with these four agents

Influenza A virus caused an epidemic with a sharp culmination in March well known among the medical profession This fact, together with a typical onset of this disease may explain why influenza patients were admitted to the hospital early in the course of disease (21 during the first three days) and also why only one third of them received antibiotic treatment before admission Further, influenza caused hospitalization mostly of elderly patients Of 42 cases all but one were patients 30 years old or more 34 of them being 50 years old or more and 17 at least 70 years It may also be mentioned that staphylococci were not a common finding in this material, whereas pneumococci, H influenzae and other Gram negative rods were frequent

Mycoplasma pneumoniae occurred sporadically during the whole period, and the infections generally did not result in a very characteristic onset of disease. One only of the 33 cases was admitted before the fourth day of disease and seven of them not until the third week or later Two-thirds had antibacterial treatment at home The age distribution was markedly different from that of influenza cases, about half of the patients being less than 30 years of age and no one had reached the age of 70

The incomplete agreement between CF test and cold agglutinin reaction is well known and it is demonstrated also in this material Of 35 patients with significantly changed or high CF titres 12 were negative in the cold agglutinin test Conversely of 34 cold agglutinin reactions 11 were not accompanied by a positive CF test This discrepancy seems to be due partly to lesser reactivity of the cold agglutinin system partly to a faster course of the latter When the first sampling was made during the first ten days of disease two only out of 15 reactions consisted of high or falling titres, as against 11 out of 19 when

sampling was begun after the tenth day

Pneumococcal infection dominated the bacterial side Serologic signs of such infection were as common in patients receiving antibacterial treatment prior to admission as in those not treated but the isolation of pneumococci was less common in treated cases A large proportion of titre shifts was in the downwards direction as stressed in a previous report (16) and recently observed also elsewhere (20) The frequency of APn reactions in pneumonic patients (42/100) did not differ significantly from that in non pneumonic patients (20/76)

H influenzae infection as indicated by AHI reactions was next to pneumococcal infection most common at admission and was evenly distributed between pneumonic and non pneumonic patients, between those treated before admission and those not treated and also between different age groups After admission H influenzae often emerged in patients treated with penicillin (16 76) and often together with AHI reactions (9 76) Also other Gram negative rods often emerged in this group (16 76) but the significance of their presence was not studied serologically Both at admission and later such Gram negative rods were mostly found in old patients generally 70 years old or more

Summary

A combined virologic and bacteriologic study has been done on 176 hospitalized patients with acute respiratory illness A serologic indication of fresh virus infection was obtained in 61 cases, eight of which also developed reactions against Mycoplasma pneumoniae (MP) In 25 other cases signs of MP infection were recorded Of these 86 cases 43 also had reactions against one (35) or two (8) bacterial species, whereas bacteria alone provoked serologic reactions in 49 cases and no antibody reactions at all were found in 41

Altogether there was evidence of a single agent in 82 cases and of two or more agents in 53

The material was characterized by a fairly high and steady frequency of MP infections, mostly in young persons and taking the form of pneumonia in about 80 % An influenza A epidemic had a sharp culmination in March The influenza patients admitted were generally more than 50 years old, and about 50 % were pneumonic

Pneumococci and H influenzae dominated the bacteriologic study Antibody titres against bacteria often decreased even during our observation time of about one month Bacterial findings were evenly distributed among all categories of patients, with only two exceptions H influenzae and other Gram-negative rods emerged especially often in patients treated with penicillin in hospital and the Gram negative rods other than H influenzae almost exclusively in very old patients

The complexity of the pattern is stressed and must be borne in mind in dealing with respiratory disease, especially in connection with therapeutic studies

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